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H. W. WILEY, CHIEF OF BUREAU.

A PRELIMINARY STUDY

OF THE

EFFECTS OF COLD STORAGE ON EGGS, QUAIL, AND CHICKENS.

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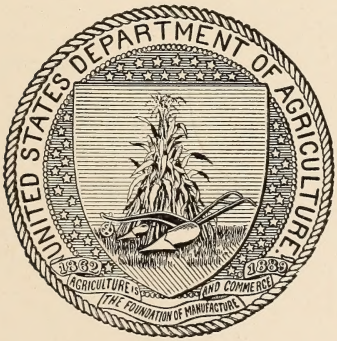

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Chemistry,
Washington, D. C., May 18, 1908.

Sir: I submit herewith for your consideration and approval the manuscript of a preliminary report on the changes in food products kept in storage, especially at low temperatures. These investigations have been carried on in harmony with the will of Congress as specifically expressed in the several appropriation bills of the last few years. The objects of the studies are fully stated in the introduction to the present report, now offered for publication as Bulletin 115 of the Bureau of Chemistry.

Respectfully,

H. W. Wiley,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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A PRELIMINARY STUDY OF THE EFFECTS OF COLD STORAGE ON EGGS, QUAIL, AND CHICKENS.

GENERAL PLAN OF THE INVESTIGATION.

In the act making appropriations for the Department of Agriculture for the fiscal year ended June 30, 1905, Congress authorized the Secretary of Agriculture, through the Bureau of Chemistry, "to investigate the adulteration of foods, condiments, beverages, and drugs, when deemed by the Secretary of Agriculture advisable, and to publish the results of such investigations when thought advisable, *and also the effect of cold storage upon the healthfulness of foods.*"

In order to carry into effect this provision of the act of Congress, a study of the effects of cold storage was organized with the following points in view:

First. To ascertain in so far as possible the kinds and character of food products kept in cold storage.

Second. To ascertain the minimum, maximum, and average length of time which such products were kept in cold storage.

Third. To ascertain the usual temperatures at which foods were held in cold storage.

(To secure an expression from the trade on these points a circular letter was prepared and forwarded to the leading cold-storage firms whose addresses could be obtained, accompanied by a blank which the persons receiving it were requested to fill out in as great detail as possible. Copies of this circular and the information obtained in reply thereto are given on pages 11 to 24.)

Fourth. To ascertain the effects of cold storage upon the organoleptic, chemical, bacteriological, and histological properties of stored foods.

In planning the different lines of investigation which should be carried on the following points were considered:

It is evident that the effect of cold-storage must be in the first place to inhibit, to a very large extent, the ordinary processes of fermentation and decay. It is well known that bodies subjected to a temperature below that of the freezing point respond very slowly and very incompletely to the action of the ordinary ferments, so that practically it may be stated that, so far as actual decay is con-

cerned, the tissues are almost as well preserved as if they had been completely sterilized and subsequently protected from any external infection. That changes, however, do go on, even in frozen bodies, is incontestable. The nature of these changes, the method by which they are effected, and the results produced are, therefore, all legitimate fields of inquiry in attempting to carry out the will of Congress. It is evident, then, that the changes which take place are of the following character:

First. There may be bacterial or enzymic action taking place in a very limited degree and yet of sufficient magnitude in the course of time to produce distinct results. Therefore, one line of investigation must evidently be directed to the ascertainment of any changes of this character.

Second. The activity of the organisms above mentioned must necessarily produce changes in chemical constitution which may affect very markedly the nutritive properties and, to that extent, the wholesomeness of the food products. Careful chemical examinations of the food products in a fresh state as they are entered at the cold-storage warehouses and also a similar examination on their withdrawal at different intervals are, therefore, necessary. The object of these examinations is, of course, to determine the nature and extent of the chemical changes produced.

Third. From the action of the above agencies it is evident also that changes in the actual construction of the tissues may be expected. These changes, which are known scientifically as histological changes, are not evident in their incipient state to the senses, and therefore can only be detected by careful microscopic examination. Special microscopical studies have therefore been made in the investigations, an account of which is to follow.

Fourth. The effects of storing certain food products, such as fowls and game birds, without removing the contents of the body cavity, as compared with changes which go on after removing such waste material, present a problem of great interest in connection with the examination. To this end, in the case of fowls and game birds, examinations were made of those stored after drawing (which is the technical term used for the removal of the entrails), and also of those stored without drawing. Birds of as nearly as possible the same age and in the same condition were stored in both the drawn and the undrawn state and removed from storage from time to time for examination.

Fifth. Among the most important of the changes which may take place in cold storage are those which relate to organoleptic properties of the foods. The color, odor, taste, tenderness, etc., of these food products have much to do with their value as food and with their effect upon health and digestion. To this end careful studies were

inaugurated relating to all of these organoleptic properties. Juries were constituted to examine food products when they were placed in cold storage, and at stated intervals thereafter, for the purpose of determining the character of the changes which may have taken place in these properties.

Sufficient progress has now been made in the work to clearly outline the exact nature of the problems which are presented, and to warrant the publication of the preliminary report. The problem, however, is one of such magnitude, and one which requires so great a length of time to study all of the conditions which have to be taken into consideration, that it can only be said at the present moment that the work has been fully inaugurated and many of the difficulties which at first presented themselves have been surmounted. A record of about two years of continued investigation has disclosed a sufficient number of valuable data to warrant their publication, not for the purpose of drawing a final conclusion respecting all the questions which have been raised, but rather for placing the matter in such a light as to clearly indicate the character and magnitude of the work still to be done.

INFORMATION FURNISHED BY COLD-STORAGE WAREHOUSEMEN.

As was stated in giving the plan of the investigation, a letter asking for the cooperation of the cold-storage warehousemen was sent out, together with a form covering the points in regard to which information was desired. The response was most satisfactory, showing every indication on the part of the trade to cooperate in the investigation, and the correspondence in condensed form is submitted as containing valuable material for comparison and study. The letter sent out by the Department read as follows:

AUGUST 15, 1904.

GENTLEMEN: An act of Congress making appropriations for the Department of Agriculture, approved April 23, 1904, authorizes the Secretary of Agriculture "to investigate the effect of cold storage upon the healthfulness of foods." This is an investigation in which the producers of foods, those who keep them in cold storage, and those who finally consume them are all equally interested.

The wholesomeness of foods is a condition which depends on a number of factors, such as soundness, freedom from products of decay, proper balance of food elements, palatability, etc.

It is well known that cold storage, at a proper temperature, improves certain food products up to a certain point. Such is the case with fresh meat, poultry, fruits, etc. There are other food products which it is well known are not improved by keeping in cold storage at any temperature, such as fish, oysters, eggs, etc. The exigencies of transportation and market conditions, however, often require that such food be kept for a time before consumption, and cold storage offers the best means of protection in such cases. It is important to know how long bodies of this kind can be kept in cold storage without materially lessening their value as food or impairing to any appreciable extent their wholesomeness. For bodies of the first class, which are improved upon keeping, there must be some definite period of storage which develops their maximum qualities, both of palatability and wholesomeness.

In carrying out the will of Congress, I desire to secure the collaboration of those engaged in the cold-storage business. It is important in the beginning of the work and in the preparation for carrying it on in detail that the general practices in regard to the period of detention for various forms of food should be known.

I have therefore submitted for your consideration a blank requesting certain information which it is hoped you can give without in any way revealing trade secrets that are regarded as private property.

In answering the questions, which I hope you will do at your earliest convenience, I shall esteem it a favor if you will suggest any lines of investigation which in your opinion may be helpful in attaining the object that Congress had in view when authorizing this investigation. All correspondence on the subject should be addressed to the Chief of the Bureau of Chemistry, in which Bureau the investigation will be conducted.

Respectfully,

WILLIS L. MOORE,
Acting Secretary.

Inclosures:
Blank form for information.
Return addressed and franked envelope.

A copy of the blank accompanying this circular, as filled out by the commissary officer of the U. S. Military Academy at West Point, is given below:

FOOD PRODUCTS IN COLD STORAGE.

1. Name and location of company: Cadet Mess, U. S. Military Academy, West Point, N. Y.
2. Principal food products kept in storage: Fresh meats of all kinds, poultry, fruit, vegetables, butter, lard, all the usual perishable food products required by a first-class educational institution.

ANIMAL PRODUCTS.

3. Fresh meats (fresh when stored).
   (a) Minimum time of storage, 2 or 3 days.
   (b) Maximum time of storage, 1 year.
   (c) Usual time of storage, 1 to 3 months' supply of beef purchased at a time.
   (d) Temperature of storage: Meats kept at 30° F., except one lot 16 hindquarters beef kept at 15° F. for experimental purposes.
4. Preserved meats (hams, shoulders, sausages, etc.).
   (a) Minimum time of storage, a week or two.
   (b) Maximum time of storage, 4 or 5 months.
   (c) Usual time of storage, 1 month.
   (d) Temperature of storage, 30° F.
5. Eggs.
   (a) Minimum time of storage: Used immediately to 1 week.
   (b) Maximum time of storage, 1 month.
   (c) Usual time of storage, 2 weeks.
   (d) Temperature of storage, 34° F.
6. Fish (fresh when stored).
   (a) Minimum time of storage: Not stored usually. Used immediately when received, though it is sometimes necessary to hold in refrigerator from 24 to 48 hours, which is done without any ill effects. Occasionally a few fish are left over from the lot and are put in a cooler where the temperature is at 15° F. These small lots have been kept in this temperature for 6 months without in any way deteriorating.

7. Oysters (fresh when stored).
   (a) Minimum time of storage: Not stored longer than 24 to 36 hours.
   (b) Maximum time of storage: ——
   (c) Usual time of storage: ——
   (d) Temperature of storage: ——
8. Poultry (fresh when stored).
   (A) Drawn.
      (a) Minimum time of storage, 1 week.
      (b) Maximum time of storage, 3 months.
      (c) Usual time of storage, 1 month.
      (d) Temperature of storage, 15° F.
   (B) Undrawn.
      (a) Minimum time of storage, 1 week.
      (b) Maximum time of storage, 3 months.
      (c) Usual time of storage, 1 month.
      (d) Temperature of storage, 15° F.
   (C) Is poultry in your house usually drawn or undrawn? Undrawn.
9. Game (birds).
   (a, b, c) Minimum time of storage: Experimental lot of 12 pairs fresh-killed partridges put in storage November, 1903. One pair used every 2 months. No deterioration noticed. Last pair now in storage appears to be all right.
   (d) Temperature of storage, 15° F.
   (e) Are they usually drawn or undrawn? Undrawn.
10. Butter.
   (a) Minimum time of storage, 1 week.
   (b) Maximum time of storage, 1 month.
   (c) Usual time of storage, 2 weeks.
   (d) Temperature of storage, 34° F.
11. Cheese.
   (a) Minimum time of storage, 1 week.
   (b) Maximum time of storage, 3 months.
   (c) Usual time of storage, 6 weeks.
   (d) Temperature of storage, 34° F.

PLANT PRODUCTS.

12. Apples and pears (summer and winter).
   (a, b, c) Minimum time of storage: Small quantities of apples and pears for immediate use occasionally stored. No effect noticed on apples when kept two weeks. Pears, however, put in green ripened in 1 week to 10 days.
   (d) Temperature of storage, 34° F.
13. Peaches.
   (a, b, c) Minimum time of storage: Not stored, except small lots for immediate use. Have kept a week, show signs of decay, and then go very fast.
   (d) Temperature of storage, 34° F.
14. Strawberries, raspberries, blackberries.
   (a, b, c) Minimum time of storage: Small lots for immediate consumption stored from time to time. Decay very fast in spite of refrigeration.
   (d) Temperature of storage, 34° F.
15. Grapes.
   (a, b, c) Minimum time of storage: Fifty crates selected Concord grapes, all perfect bunches, no overripe, immature, or bruised fruit left on the bunches. They became musty and dried, falling off the stems; experiment unsuccessful. Were stored 2 months.
   (d) Temperature of storage, 34° F.

16. Oranges and lemons.
    (a, b, c, d) Minimum time of storage: Oranges have been stored for 1 month and lemons have been kept 3 months with good results, when the fruit was perfectly sound and dry when stored. When wet or decayed when stored, cold storage did not prevent the spread of further decay.
17. Green peas and beans.
    Not stored.
18. Tomatoes.
    (a) Minimum time of storage, a few days.
    (b) Maximum time of storage, 2 weeks.
    (c) Usual time of storage, 1 week. Very successful with these.
    (d) Temperature of storage, 34° F.
19. Cucumbers.
    (a) Minimum time of storage, 1 week.
    (b) Maximum time of storage, 1 month.
    (c) Usual time of storage, 2 weeks. Very successful with these.
    (d) Temperature of storage, 34° F.
20. Celery.
    (a) Minimum time of storage, a few days.
    (b) Maximum time of storage, 3 weeks.
    (c) Usual time of storage, 1 week.
    (d) Temperature of storage, 34° F. Very successful with this.
21. Cabbage.
    Not stored.
22. Potatoes.
    Not stored.

MISCELLANEOUS PRODUCTS.

[No statements.]

A large number of replies was received in response to this circular, and the information given has been compiled in tabular form as follows:

Tabulation of data furnished by warehousemen.

(A) ANIMAL PRODUCTS.

[Representing 43 firms.]

FRESH MEATS.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Weeks.		° *F.*	
One	Two.	Below 0	Three.
Two	Seven.	0 to 9	Twelve.
Three	One.	10 to 24	Ten.
Four	Six.	25 to 31	Eight.
Six	Two.	32 to 40	Six.
Eight	Four.		
Over eight	Twelve.		

Average maximum time	days	201
Average minimum time	do	17
Average usual time	do	58
Average temperature	°F	12.5

Remarks.—In many cases the firms differentiate two classes of fresh stored meats, namely, frozen and unfrozen. The first named is kept at lower temperatures (−22° to 10°) and for longer periods of time than the unfrozen (kept at 28° to 40° F.).

Tabulation of data furnished by warehousemen—Continued.

(A) ANIMAL PRODUCTS—Continued.

PRESERVED MEATS.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Months.		°F.	
One	Seven.	Below 0	One.
Two	Three.	0 to 9	Two.
Three	Nine.	10 to 24	
Four	Two.	25 to 31	Seven.
Five	One.	32 to 40	Fifteen.
Six	Two.	Over 40	One.
Seven			
Eight	Two.		

Average maximum time	days	193
Average minimum time	do	34
Average usual time	do	84
Average temperature	°F.	29.3

Remarks.—In many cases the firms differentiate two classes of preserved stored meats, cured and uncured, and prescribe different conditions for their storage. Under the seven firms for whom one month is stated as the average time held are included those giving shorter periods of time. This reduces the average, which is really just three months.

EGGS.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Months.		°F.	
One	Six.	Below 0	
Two	One.	0 to 9	One.
Three	Five.	10 to 24	
Four		24 to 31	Twenty-three.
Five	Three.	32 to 40	Eleven.
Six	Eleven.	Over 40	
Seven	Four.		
Eight	Seven.		

Average maximum time	days	256
Average minimum time	do	49
Average usual time	do	159
Average temperature	°F.	30.3

Remarks.—Under the six firms for which one month is stated as the average time held are included shorter periods of time, reducing the average time, which is really about six months.

FISH.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Months.		°F.	
One	Two.	Below 0	One.
Two	Four.	0 to 9	Eight.
Three	Three.	10 to 24	Thirteen.
Four	Four.	25 to 31	One.
Five	Three.	32 to 40	One.
Six	Six.	Over 40	
Seven			
Eight	One.		

Average maximum time	days	229
Average minimum time	do	34
Average usual time	do	133
Average temperature	°F.	11.3

Remarks.—Several firms state that they freeze the fish at once at temperatures from −15° F. to 5° F., then carry at the temperatures given.

Tabulation of data furnished by warehousemen—Continued.

(A) ANIMAL PRODUCTS—Continued.

OYSTERS.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Weeks.		° *F.*	
One	Four.	Below 0	
Two	Three.	0 to 9	One.
Three		10 to 24	One.
Four		25 to 31	Three.
Five		31 to 40	Seven.
Six		Over 40	
Seven			
Eight	One.		
More than eight	One.		

Average maximum time	days	50
Average minimum time	do	14
Average usual time	do	29
Average temperature	°F	27.6

REMARKS.—One Alaska firm keeps oysters a much longer period than any firm in this country.

POULTRY (DRAWN).

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Months.		° *F.*	
One	Two.	Below 0	One.
Two	Four.	0 to 9	Seven.
Three	Four.	10 to 24	Seven.
Four	Three.	25 to 31	Two.
Five	Two.	32 to 40	Two.
Six	One.	Over 40	
Seven			
Eight			
More than eight	One.		

Average maximum time	days	219
Average minimum time	do	34
Average usual time	do	112
Average temperature	°F	12.4

REMARKS.—Several firms who handle undrawn poultry state that they do not handle drawn poultry, and one firm says experience has taught them that the undrawn keeps better than the drawn.

POULTRY (UNDRAWN).

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Months.		° *F.*	
One	Two.	Below 0	Four.
Two	Four.	0 to 9	Nine.
Three	Three.	10 to 24	Sixteen.
Four	Nine.	25 to 31	Two.
Five	Four.	32 to 40	Two.
Six	Four.	Over 40	
Seven	One.		
Eight	One.		
More than eight	Two.		

Average maximum time	days	256
Average minimum time	do	32
Average usual time	do	131
Average temperature	°F	11.7

REMARKS.—In answer to the question: Is the poultry in your house usually drawn or undrawn, seven firms replied "drawn" and twenty-six "undrawn." Sixteen of the latter stated that it was always undrawn. Three answered that they stored both, without stating any preference.

Tabulation of data furnished by warehousemen—Continued.

(A) ANIMAL PRODUCTS—Continued.

GAME (BIRDS).

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Months.		°*F.*	
One	One.	Below 0	One.
Two	Six.	0 to 9	Six.
Three	Two.	10 to 24	Thirteen.
Four	Four.	25 to 31	Two.
Five	Three.	32 to 40	Two.
Six		Over 40	
Seven			
Eight			
More than eight	Four.		

Average maximum time	days..	275
Average minimum time	do....	29
Average usual time	do....	133
Average temperature	°F..	16

Remarks.—Two firms stated that they were controlled by the game laws as to time of storage. One stated that all game stored should be drawn, since it is shot.

In reply to the question whether they were usually drawn or undrawn, *three* firms stated that they were usually drawn, and *fifteen*, usually or always undrawn. One firm said they stored both kinds, equally.

BUTTER.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Months.		°*F.*	
One	Two.	Below 0	Eight.
Two	One.	0 to 9	Fourteen.
Three	Three.	10 to 24	Thirteen.
Four	Five.	25 to 31	Three.
Five	Seven.	32 to 40	Two.
Six	Twelve.	Over 40	
Seven	Three.		
Eight	Five.		
More than eight			

Average maximum time	days..	301
Average minimum time	do....	39
Average usual time	do....	156
Average temperature	°F..	8

CHEESE.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Months.		°*F.*	
One	One.	Below 0	
Two	One.	0 to 9	One.
Three	Five.	10 to 24	Two.
Four	Six.	25 to 31	Six.
Five	Seven.	32 to 40	Twenty-three.
Six	Nine.	Over 40	
Seven	Four.		
Eight	One.		
More than eight	One.		

Average maximum time	days..	292
Average minimum time	do....	42
Average usual time	do....	146
Average temperature	°F..	32.5

Tabulation of data furnished by warehousemen—Continued.

(B) VEGETABLE PRODUCTS.

[Representing 43 firms.]

APPLES AND PEARS.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Months.		°F.	
One	Five.	Below 0	
Two	Three.	0 to 9	One.
Three	Four.	10 to 24	
Four	Eleven.	25 to 31	Thirteen.
Five	Eight.	32 to 34	Eighteen
Six	Six.	35 to 40	Four.
Seven	One.	Over 40	
Eight			
More than eight			

Average maximum time	days	188
Average minimum time	do	34
Average usual time	do	122
Average temperature	°F.	31.5

Remarks.—Many stated that pears are not so successfully held in cold storage as apples, and gave the limit as 60 to 90 days, at 32° F.

PEACHES.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Weeks.		°F.	
One	Two.	Below 0	
Two	Eight.	0 to 9	
Three	Three	10 to 24	
Four	Two.	25 to 31	
Five		32 to 34	Thirteen.
Six	One.	35 to 40	Five.
Seven	One.	Over 40	
Eight			
More than eight			

Average maximum time	days	39
Average minimum time	do	7
Average usual time	do	19
Average temperature	°F.	34.4

STRAWBERRIES, RASPBERRIES, AND BLACKBERRIES.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Weeks.		°F.	
One	Twelve.	Below 0	
Two	One.	0 to 9	
Three	Two.	10 to 24	
Four		25 to 31	
Five		32 to 34	Six.
Six		35 to 40	Ten.
Seven		Over 40	One.
Eight			
More than eight			

Average maximum time	days	16
Average minimum time	do	3
Average usual time	do	7
Average temperature	°F.	36

Tabulation of data furnished by warehousemen—Continued.

(B) VEGETABLE PRODUCTS—Continued.

GRAPES.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Months.		°F.	
One	Three.	Below 0	
Two	One.	0 to 9	
Three	Three.	10 to 24	
Four	Four.	25 to 31	
Five		32 to 34	Eight.
Six	One.	35 to 40	Eight.
Seven	One.	Over 40	
Eight	Two.		
More than eight	One.		

Average maximum time	days..	79
Average minimum time	do....	12
Average usual time	do....	32
Average temperature	°F..	34

ORANGES AND LEMONS.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Months.		°F.	
One	Six.	Below 0	
Two	Seven.	0 to 9	
Three	Six.	10 to 24	
Four	Three.	25 to 31	
Five		32 to 34	Five.
Six		35 to 40	Eighteen.
Seven		Over 40	Four.
Eight			
More than eight			

Average maximum time	days..	108
Average minimum time	do....	26
Average usual time	do....	66
Average temperature	°F..	37.7

REMARKS.—Many distinguish between oranges and lemons, storing lemons at a higher temperature and for longer periods than oranges.

GREEN PEAS AND BEANS.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Weeks.		°F.	
One	Five.	Below 0	
Two	Four.	0 to 9	
Three		10 to 24	
Four		25 to 31	
Five		32 to 34	Three.
Six		35 to 40	Seven.
Seven		Over 40	
Eight			
More than eight			

Average maximum time	days..	19
Average minimum time	do....	3
Average usual time	do....	7
Average temperature	°F..	34

Tabulation of data furnished by warehousemen—Continued.

(B) VEGETABLE PRODUCTS—Continued.

TOMATOES.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Weeks.		°*F.*	
One	Four.	Below 0	
Two	Three.	0 to 9	
Three		10 to 24	
Four	One.	25 to 31	
Five		32 to 34	Six.
Six	One.	35 to 40	Five.
Seven		Over 40	
Eight			
More than eight			

Average maximum time	days..	31
Average minimum time	do....	3
Average usual time	do....	16
Average temperature	°F..	36

CUCUMBERS.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Weeks.		°*F.*	
One	Five.	Below 0	
Two	Four.	0 to 9	
Three		10 to 24	
Four	One.	25 to 31	One.
Five		32 to 34	Three.
Six		35 to 40	Nine.
Seven		Over 40	
Eight			
More than eight			

Average maximum time	days..	36
Average minimum time	do....	5
Average usual time	do....	11
Average temperature	°F..	28

CELERY.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Weeks.		°*F.*	
One		Below 0	
Two	Four.	0 to 9	
Three	Four.	10 to 24	
Four	Five.	25 to 31	
Five	One.	32 to 34	Twelve.
Six	One.	35 to 40	Eight.
Seven		Over 40	
Eight	Three.		
More than eight	One.		

Average maximum time	days..	71
Average minimum time	do....	11
Average usual time	do....	35
Average temperature	°F..	34.4

Tabulation of data furnished by warehousemen—Continued.

(B) VEGETABLE PRODUCTS—Continued.

CABBAGE.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Weeks.		° *F.*	
One		Below 0	
Two	One.	0 to 9	One.
Three	Two.	10 to 24	
Four	Two.	25 to 31	
Five	One.	32 to 34	Ten.
Six	Two.	35 to 40	Four.
Seven		Over 40	
Eight	Three.		
More than eight	Five.		

Average maximum time	days..	96
Average minimum time	do....	24
Average usual time	do....	57
Average temperature	°F..	32.2

POTATOES.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Months.		° *F.*	
One	Four.	Below 0	
Two	One.	0 to 9	
Three	Eight.	10 to 24	
Four	Three.	25 to 31	
Five		32 to 34	Six.
Six	One.	35 to 40	Eleven.
Seven		Over 40	
Eight			
More than eight			

Average maximum time	days..	133
Average minimum time	do....	28
Average usual time	do....	71
Average temperature	°F..	35

(C) MISCELLANEOUS.

CONDENSED MILK.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Months.		° *F.*	
One	Two.	Below 0	
Two		0 to 9	
Three		10 to 24	
Four		25 to 31	Two.
Five		32 to 40	Two.
Six	Two.	Over 40	
Seven			
Eight			
More than eight	One.		

Average maximum time	days..	165
Average minimum time	do....	40
Average usual time	do....	123
Average temperature	F°..	32.3

Tabulation of data furnished by warehousemen—Continued.

(C) MISCELLANEOUS—Continued.

NUTS.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Months.		°*F.*	
One		Below 0	
Two		0 to 9	
Three		10 to 24	
Four	One.	25 to 31	One.
Five	One.	32 to 40	Two.
Six	One.	Over 40	
Seven			
Eight			
More than eight	One.		

Average maximum time	days..	180
Average minimum time	do....	30
Average usual time	do....	155
Average temperature	°F..	31.6

MOLASSES.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Months.		°*F.*	
One		Below 0	
Two		0 to 9	
Three		10 to 24	
Four	One.	25 to 31	
Five		32 to 40	Two.
Six	One.	Over 40	
Seven			
Eight			
More than eight	Two.		

Average maximum time	days..	180
Average minimum time	do....	60
Average usual time	do....	150
Average temperature	°F..	34.5

DRIED FRUITS.

Length of time in storage.	Number of firms averaging this time.	Temperature at which held.	Number of firms averaging this temperature.
Months.		°*F.*	
One		Below 0	
Two		0 to 9	
Three	One.	10 to 24	
Four	Two.	25 to 31	One.
Five	One.	32 to 34	Three.
Six	Two.	35 to 40	Two.
Seven		Over 40	
Eight			
More than eight			

Average maximum time	days..	180
Average minimum time	do....	30
Average usual time	do....	140
Average temperature	°F..	33.3

PRODUCTS REPORTED BY ONE FIRM ONLY.

Rabbits:

Minimum time of storage, 1 week.

Maximum time of storage, 2 months.

Usual time of storage, 2 weeks.

Temperature of storage, short storage, 32° F.; long, 17° F.

Fresh milk:
Minimum time of storage, in short storage of 1 or 2 weeks.
Temperature of storage, 33° to 34° F.
Buckwheat flour:
Minimum time of storage, 1 month.
Maximum time of storage, 6 months.
Usual time of storage, 6 months.
Temperature of storage, 32° F.
Horse radish:
Minimum time of storage, 1 month.
Maximum time of storage, 6 months.
Usual time of storage, 3 months.
Temperature of storage, 33° to 35° F.
Macaroni:
Usual time of storage, from 1 to 4 months.
Temperature of storage, 33° F. dry.
Oleomargarine:
Maximum time of storage, 6 months.
Usual time of storage, 3 to 6 months.
Temperature of storage, 0° F.
Pickles:
Usual time of storage, 3 to 6 months.
Temperature of storage, 32° to 34° F.
Watermelons:
Maximum time of storage, 30 days.
Usual time of storage, 10 days.
Temperature of storage, 40° F.
Pickled salmon:
Minimum time of storage, 1 month.
Maximum time of storage, 6 months.
Temperature of storage, first 3 weeks, 28° to 30°; balance of time, 34° F.

In some cases letters were written to accompany the blanks. A cold-storage company from the extreme Northwest writes as follows:

* * * Judging from your letter you are in quest of data in relation to refrigerated products, not frozen meats, etc., such as we handle. Our business is that of wholesale and retail meat dealers. We store frozen animal and fish products and retail to the trade and to the consumer direct. We do not handle refrigerated meats at all; by that I mean meats that are held at a temperature but slightly above the freezing points for such products; all our stuff is hard-frozen, and is kept so until finally delivered to the consumer. This is made necessary owing to transportation conditions which necessitate our laying in a sufficient stock to last through the winter months during the time of the open season in this country; i. e., June, July, August, and September. We can not hold "cooled" meats that long, about seven months, and so we freeze everything "hard." That chilling meats improves them I firmly believe, but that freezing them adds to their quality I seriously question.

During the summer months we furnish fresh-killed meats to the trade, but from October until the middle of June we handle nothing but the frozen products. We have had meats in storage as long as 14 to 16 months, at a temperature ranging from 14° F. in the summer, to 10° to 20° F. below zero during the winter, and they have been

furnished to the trade in apparently as good a condition as when they were first put in storage. We have no trouble in holding meats for eight to ten months in a hard-frozen condition, and the quality is eminently satisfactory. Very rarely do we have any complaints from the trade; once or twice during the season, perhaps. Given a stable temperature of about 15° F., with no more fluctuation than 2°, I believe meats can be held hard-frozen indefinitely, say several years, without further deterioration in quality other than that which transpires when same are first frozen. But in this country, where the outside temperature ranges from zero to as much as 60 below at times, it is impossible to keep an equable degree of cold, as the outside temperature is bound to have an effect on the cold-storage chambers, producing in them lowering of the temperature, and this excessive coldness has a tendency to take more or less of the life out of the meats and renders them slightly dryer than otherwise if kept for any length of time, say more than a year and a half. * * *

A cold storage warehouseman of many years' experience makes the following statement:

* * * My present cold-storage occupation will not be of much use to you, as we do not handle any goods first-handed. I established one of the first cold-storage plants in the country and have had no end of experience, especially in the meat line. I have slaughtered at home, bought at Chicago both alive and dressed, and cut up all grades and qualities. As a matter of trade cold storage is required to do this. After 48 hours it never improves the taste of any goods or their health-giving nutriment. I have eaten meat I have kept a year; while tender, it lost a large part of its taste. I do not consider it wholesome. I could fill 20 pages with experience, but it would not help you. There are so many different qualities in the same kinds that it would be hard to set a limit.

Another storage company sends, in addition to the blank filled out, the following information:

* * * We do not exactly understand whether the minimum and maximum time of storage is intended to show the customary times which these products remain in storage, or whether it is the intention to show the time certain goods might attain their best condition, and the time when they would be expected to begin to deteriorate. We presume, however, that it is the former, and have made our answers on that basis, assuming to a large extent that the withdrawals of these goods indicate the latter.

Market conditions always enter into the matter also. Taking, for instance, fresh meats, we handle a great deal which is taken out almost as soon as thoroughly frozen, but the great bulk of it will remain for some time. We do not consider, however, that it can be carried satisfactorily over three months, as it will begin to show more or less dryness, etc.

As to fish, it is not uncommon for us to carry them one year or longer, but we consider them in nearly all cases beginning to deteriorate after three or four months, unless they are redipped in water, thus forming a thin coat of ice over them which will protect them from deterioration almost indefinitely, so long as this process is renewed from time to time. Practically, however, this can not be done often on account of the cost, which would become an important factor, and we merely refer to this as indicating what might be done.

As to butter, where we show the minimum time of ten days you will understand that this refers to butter which is put in refrigerating rooms for the purpose of carrying it for a short time when the market may be overloaded or for some similar reason. For June creameries intended for use during the following winter the temperature is held from 5° to 10° above and also to 5° below zero. It is now assumed by the trade that the best results in carriage are obtained at the lower temperature. * * *

INCEPTION OF THE WORK.

Among the most important of the foods so far examined on which the work is sufficiently advanced to warrant publication are chickens, quail, milk, and eggs. The conditions under which the eggs were purchased and stored are given under the appropriate caption on page 27. The preliminary study on milk, conducted by M. E. Pennington, has been reported in the Journal of Biological Chemistry, April, 1908, and will not be reprinted in this report, save for the conclusions drawn therefrom, which will be found in the summary on page 101.

The chemical analysis of the eggs was made by F. C. Cook and the microscopic examination by B. J. Howard. The bacteriological examination of the eggs, quail, and chickens conducted at Washington was made by G. W. Stiles, and the study of market cold-storage chickens, covering chemical, bacteriological, and histological changes, was conducted at Philadelphia by M. E. Pennington.

In the tests made at Washington a large number of chickens of as nearly as possible the same age and condition were secured in a perfectly fresh state, slaughtered, and prepared for storage by the removal of the feathers, and packed six in a box, as is the custom in placing samples in storage. The fowls were placed in cold storage at a temperature from 2° to 8° F.—that is, frozen solid—half being drawn and half left in the undrawn state. A sufficient number of these chickens were dressed, cooked, and subjected to the judgment of the jury in regard to the quality and character of the flesh. Another lot, representative in character, was selected for examination.

A large number of quail in like manner were secured by special arrangement with the North Carolina game warden, so that the birds might be shipped directly to Washington on the date they were killed. In this way the exact length of time which had elapsed since their slaughter was known. These birds were treated in the same manner as the chickens, having been tested and placed in cold storage, half drawn and half undrawn. At stated intervals, usually of about three months, samples of these storage products were withdrawn and subjected to the same examination as has just been indicated. Fresh chickens, as nearly of the same size and character as those in cold storage as could be secured, were always prepared with these samples. When these chickens were cooked and presented to the jury they were numbered or indicated in some way so that no member of the jury knew which lot was the fresh chickens, which the drawn chickens in storage, and which the undrawn chickens in storage. Each individual member of the jury was requested to determine for himself the character, odor, color, and taste, to enter the same in a notebook, and to determine from the results of his notes and his experience which he considered to be the best for eating purposes of the samples presented to him. The members of the jury were not allowed to consult among

themselves until their final verdict had been rendered. The data thus noted were collected and summarized in order to determine whether or not there had been any consensus of opinion respecting the important points to be considered.

A careful organoleptic examination of the chickens was also made before cooking, but in this case, inasmuch as the chickens were seen in the state in which they had been removed from storage, it was, of course, perfectly easy for each inspector to determine which was the fresh, which the drawn, and which the undrawn bird. These inspections consisted in a careful study of the external appearance of the chicken, and also of the body cavity, in both the drawn and the undrawn chickens after the latter had been dressed for cooking. It is perfectly easy in such cases to distinguish between a fresh and a cold-storage chicken by the external and internal appearances. The odor, external and internal, was also noted. The recession of the eye of the cold-storage chicken, the changes of the color of the blood, the change to a certain extent of the color of the interior of the bird and its flesh, the development of blueness around the muscles of the legs, the ease with which the flesh is detached from the bone, the odor of the bird, the shriveled appearance of its skin—all are highly indicative of the changes which have taken place. It may be said that a careful inspection of cold-storage fowls, whether drawn or undrawn, before cooking would do much to destroy any appetite which might otherwise have been manifested for these birds when cooked. The process of cooking, however, does very much to eliminate the differences between the fresh and the cold-storage fowls. To such an extent is this true that at the end of the first three months there were very great differences of opinion among the jury regarding the identity of the samples tested.

It is generally considered by connoisseurs that a certain delay between the killing and the consumption of chickens and game birds is necessary to develop the proper tenderness and flavor of the flesh. This is a problem, however, which can not be discussed very extensively at this point, since in the old-fashioned way the hanging of poultry and birds for a certain length of time was considered necessary for the purpose of "ripening," so called. They were not subjected, as a rule, to as low a temperature as that found in cold-storage warehouses. The common method, especially in Germany, was to hang the Christmas goose or turkey from a back window for ten days or two weeks previous to the serving of the meal, and thus it was subjected sometimes to temperatures below freezing and sometimes to those above. At the same time the birds thus exposed were hanging in the free air and were relieved of the danger of being left in the stagnant air of a storage warehouse. It is said that one of the best methods of sending the wild duck across the water in the winter is to nail them to the mast of a slow-going vessel. Their exposure in this way is said to place them

in the best possible condition for consumption on their arrival on the other side. It does not follow that the detention of birds in a closed cold-storage compartment below the freezing point for the same or greater periods of time will produce the same effect.

I. COLD-STORAGE EGGS.[a]

PLAN OF WORK.

The eggs for this investigation were secured from a reliable local dealer, who stated that all were laid on May 23, 1906, and they were placed in storage on the following day. The wood of the crates was thoroughly seasoned and, because of absorption of odors by the eggs, they were made of white wood, gum, or other odorless wood. The strawboard supporting the eggs also affects the odor somewhat. All of the crates were new.

The eggs were divided into 10 lots of one dozen each for storage and the weight of each dozen taken. One dozen was reserved for immediate analysis; the others were placed in a storage room having a temperature of 33° F. The weights per dozen on May 24, 1906, were as follows:

	Grams.		Grams.
Dozen No. 2	669	Dozen No. 7	647
Dozen No. 3	639	Dozen No. 8	647
Dozen No. 4	648	Dozen No. 9	612
Dozen No. 5	670	Dozen No. 10	611
Dozen No. 6	641	Dozen No. 11	623

At certain intervals, as indicated in the table, a dozen eggs were removed for analysis. Four of these were examined chemically in a raw state and four were hard boiled; two were used in an attempt to determine volatile sulphur, but as only minute traces were found in either fresh or cold-storage eggs this estimation was excluded. One or two eggs were referred to the bacteriologist for examination, whose findings are given elsewhere in this report. Samples were sent also to the microscopist. The following determinations were made on whites and yolks, separately, of both boiled and unboiled eggs,[b] the weight of the material having been determined before and after cooking: Moisture, ash, ether extract, total sulphur, total phosphorus, and lecithin phosphorus. The total nitrogen was determined, and also the nitrogen in the form of coagulable proteids, proteoses and peptones, and amido bodies on the boiled samples.

The moisture, ash, ether extract, total sulphur, and total phosphorus were determined by the methods of the Association of Official Agricultural Chemists.[c] The lecithin phosphorus was determined

[a] Chemical analysis made by F. C. Cook.

[b] The separation of the whites and yolks of the unboiled eggs was not satisfactory, and therefore the results were recalculated to the whole egg. See table, page 31.

[c] U. S. Dept. Agr., Bureau of Chemistry, Bul. 107, Revised.

in the same sample in which the ether extract was estimated. The sample was extracted eight hours with absolute alcohol directly into the ether extract flask and the total phosphorus estimated in the ether-alcohol extract, which is computed as the lecithin phosphorus. The total nitrogen was determined by the official Kjeldahl-Gunning method [a] and the amido bodies by the tannin-salt method.[b] The proteose and peptone figures were determined by difference, the uncoagulable nitrogen minus the amido nitrogen giving the proteose and peptone nitrogen. The reaction which was determined in some of the samples was obtained by titrating a dilute solution of the whites and yolks with decinormal sodium hydrate or decinormal sulphuric acid, using phenolphthalein as indicator. The coagulable proteid figures for the boiled samples were obtained by boiling 20 grams of the sample with 300 cc of water for three minutes, estimating the nitrogen in an aliquot of the filtrate, and deducting the figure thus obtained from the total nitrogen.

The variations in weight referable to storage are given in the table.

Loss of weight per dozen eggs placed in cold storage May 24, 1906.

Time of storage.	Original weight.	Final weight.	Loss in weight.	
1906.	*Grams.*	*Grams.*	*Grams.*	*Per cent.*
September 7, 3.5 months	623	602	21	3.4
1907.				
January 7, 7.5 months	611	578	33	5.4
June 17, 12.6 months	612	565	47	7.7
October 16, 16.6 months	647	582	65	10.0
1908.				
January 14, 19.6 months	647	582	65	10.0

In all of the analyses of cold-stored eggs, the samples were removed from the cold-storage rooms in the afternoon and immediately placed in the ice box at the Bureau of Chemistry, where they stood overnight, and the analysis was begun the following morning. The eggs, therefore, did not stand at room temperature for any length of time. What would have been the effect on the eggs of room temperature for two or three days, as is often the case in the household, these experiments do not show.

PHYSICAL CHARACTERISTICS.

Though the eggs had been kept at 1° F. above freezing, they showed a marked tendency to sweat when transferred to ordinary temperatures, the shells becoming very wet and also more brittle. After breaking the shell and keeping at room temperature for one day, the odor of the eggs in storage for 3.5 months was not unpleasant,

[a] U. S. Dept. Agr., Bureau of Chemistry, Bul. 107, Revised.
[b] J. Amer. Chem. Soc., 1906, *28:* 1485.

though it differed from the fresh. At the expiration of 7.5 months the difference was more marked, and after 12.6 months it had so increased that it was characteristic of cold-stored eggs, and can only be described as such. After 16.6 months a musty odor was noticed as soon as the egg was opened.

The integrity of whites and yolks was preserved at the end of 3.5 months; at the end of 7.5 months considerable difficulty was experienced in separating the whites and yolks of the unboiled eggs, though in the boiled eggs this was accomplished readily. When storage had been maintained for 19.6 months it was found impossible to separate the yolks and whites of the raw eggs, the latter invariably contaminating the former. The whites, which had been gradually losing their thick, gelatinous condition and becoming more and more watery, were now very thin and showed no tendency to adhere to the yolk or to cohere. The yolk membrane was so tender that when separated from the white it ruptured, though most carefully manipulated. When cooked, the whites were rather pink and the yolks were much darker in color than were those of the fresh eggs. The consistency of the boiled yolk varied from that of the fresh yolk when storage had been maintained for 16.6 months, the mealy character being much reduced. Neither did the unboiled yolks float on water, as they do when fresh.

The physical variations from the normal are confirmed by the changes in weight of the eggs when cold stored as compared with the fresh. When fresh eggs are boiled, a loss in weight occurs, while storage eggs gain on boiling. Apparently the whites lose more water than do the yolks, and, consequently, gain more when boiled. The boiled yolks when fresh contain less than 50 per cent of water; when cold-stored this percentage is increased, the figure reaching 64 per cent in the last examination.

CHEMICAL ANALYSIS.

DISCUSSION OF DATA.

The average ash content of all the samples of fresh eggs is slightly lower than that of the cold-storage eggs, and this is not entirely explained on the basis of the decreased moisture content of the cold-storage eggs, as may be seen from the table calculated to a water-free basis.

The total sulphur and the total phosphoric acid in the boiled yolks do not show any progressive change for the eggs which have been in storage for a period of 19 months. After $3\frac{1}{2}$ months' storage the percentage of total phosphorus as lecithin phosphorus in the boiled storage yolks is lower than in the boiled yolks of fresh eggs. It is probable, therefore, that the organic phosphorus compound of the egg yolk is being broken down into inorganic phosphorus.

The nitrogenous substances present in the boiled eggs examined show a change in the amount of coagulable proteid, which decreases during the storage of the eggs. The analytical data on the separated whites and yolks in a cooked state show a rise in the water content of the yolks of the storage eggs and a decrease in this constituent in the case of the whites.

The amido-nitrogen figures show a tendency to decrease in the case of the cold-storage eggs, but this effect is not uniform, and consequently no definite conclusion can be drawn as to a change in amount of amido nitrogen in cold-storage eggs. The proteose and peptone nitrogen content of cold-storage eggs shows a tendency to increase.

The reaction of the yolks, both fresh and those stored for 19.8 months, were slightly acid, while in both cases the whites gave an alkaline reaction. This acid reaction of the yolks of the storage eggs was about one-half as strong as in the case of the fresh eggs.

CONCLUSIONS.

Eggs in storage for one year show a loss of weight equivalent to 10 per cent of the total weight, which loss is largely water from the whites. Eggs after storage for 16.5 months lose their power of cohesion and emit a characteristic musty odor a few hours after opening. A lowering of the amount of coagulable proteid of the boiled sample is indicated, as well as a change in reaction and a lower percentage of lecithin phosphorus in the storage eggs. An increase in the lower nitrogen bodies, proteoses and peptones, accompanies the decrease of coagulable nitrogen in the boiled samples of storage eggs, while there is apparently a tendency for the amido bodies to decrease.

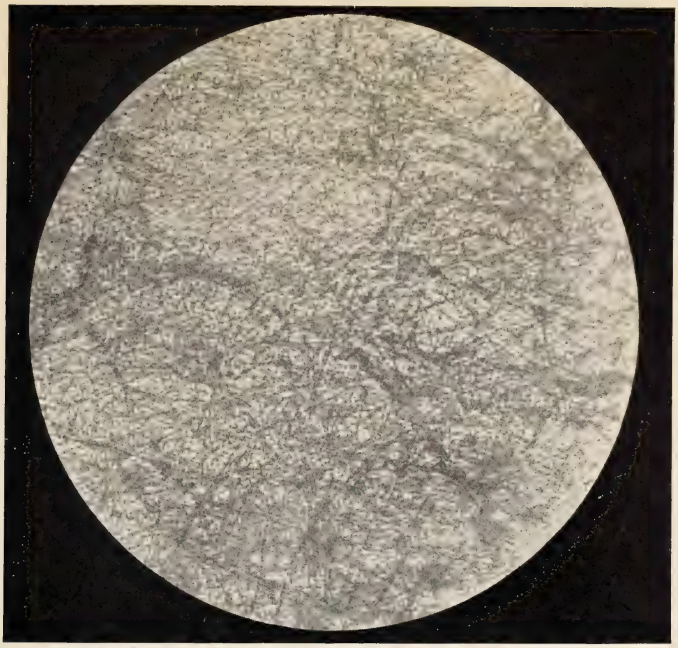

FIG. 1.—OUTER SHELL MEMBRANE OF FRESH EGG (×150).

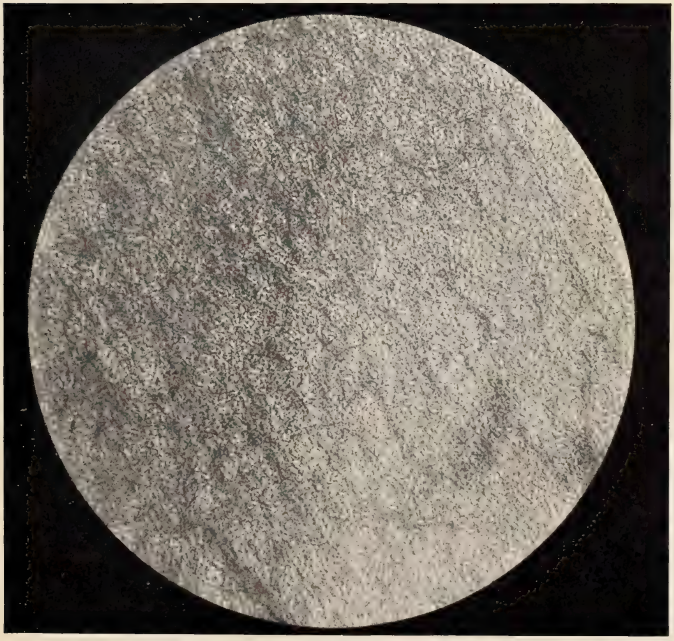

FIG. 2.—INNER SHELL MEMBRANE OF FRESH EGG (×150).

Analysis of entire egg, unboiled, fresh, and cold storage.[a]

WET BASIS.

Serial No.	Date.	Description of sample.	Solids.	Ash.	Ether extract.	Total sulphur.	Total P_2O_5.	Total nitrogen.
			Per cent.	*Per ct.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
16816, 16817	May 25, 1906	Fresh eggs	25.28	0.69	.....	.....	.....	1.90
AP140, AP141	Jan. 17, 1908	.....do	27.70	.95	12.89	0.24	0.67	2.01
17160, 17159	Sept. 7, 1906	Storage 3½ mos	27.29	.97	.....	.16	.39	1.68
17680C, 17680D	Jan. 7, 1907	Storage 7½ mos	33.56	.91	.....	.13	1.00	2.10
17919C, 17919D	June 17, 1907	Storage 12⅔ mos	24.53	.91	.....	.18	.58	2.09
AP3, AP4	Oct. 17, 1907	Storage 16⅔ mos	31.62	1.08	12.69	.20	.74	2.23
AP144, AP125	Jan. 17, 1908	Storage 19⅔ mos	29.78	1.00	12.15	.27	.70	2.26

CALCULATED TO A WATER-FREE BASIS

Serial No.	Date.	Description of sample.	Ash.	Ether extract	Total sulphur.	Total P_2O_5.	Total nitrogen.
			Per ct.	*Per ct.*	*Per cent.*	*Per cent.*	*Per ct.*
16816, 16817	May 25, 1906	Fresh eggs	2.73	.....	.....	.....	7.51
AP140, AP141	Jan. 17, 1908	.....do	3.42	46.54	0.87	2.42	7.26
17160, 17159	Sept. 7, 1906	Storage 3½ mos	3.55	.....	.59	1.43	6.16
17680C, 17680D	Jan. 7, 1907	Storage 7½ mos	2.71	.....	.39	2.98	6.26
17919C, 17919D	June 17, 1907	Storage 12⅔ mos	3.71	.....	.73	2.36	8.52
AP3, AP4	Oct. 17, 1907	Storage 16⅔ mos	3.42	40.13	.63	2.34	7.05
AP144, AP145	Jan. 17, 1908	Storage 19⅔ mos	3.36	40.80	.91	2.35	7.59

[a] Whites and yolks analyzed separately and results calculated for total egg.

Analysis of boiled yolks of fresh and cold-storage eggs.

WET BASIS.

Serial No.	Date.	Description of sample.	Moisture.	Ash.	Ether extract.	Total sulphur.	Total P_2O_5.	Lecithin P_2O_5.	Nitrogen of egg present as—				
									Total.	Coagulable.	Uncoagulable.	Proteoses and peptones.	Amido.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
AP6	Oct. 17, 1907	Fresh eggs	48.74	1.08	30.43	0.21	1.31	0.90	2.61	2.412	0.198	0.090	0.108
AP143	Jan. 17, 1908	do	45.39	1.28	33.89	.23	1.47	1.025	2.63	2.546	.084	.008	.076
17158	Sept. 7, 1906	Storage 3½ mos	49.02	1.54	31.38	.20	1.10	.55	2.50	2.37	.130	.064	.066
17680B	Jan. 7, 1907	Storage 7½ mos	50.49	1.34	36.40	.16	1.90	.....	2.56	2.424	.136	.069	.067
17919B	June 17, 1907	Storage 12⅜ mos	52.09	1.41	29.66	.173	1.293	.831	2.39	2.199	.191	.112	.079
AP8	Oct. 17, 1907	Storage 16⅝ mos	64.06	1.92	24.82	.20	1.35	.84	2.315	1.117	1.198	1.144	.054
AP147	Jan. 17, 1908	Storage eggs 19¾ mos	50.82	1.66	29.19	.22	1.28	.83	2.545	.865	1.680	1.654	.026

CALCULATED TO A WATER-FREE BASIS.

Serial No.	Date.	Description of sample.	Ash.	Ether extract.	Total sulphur.	Total P_2O_5.	Lecithin P_2O_5.	Total P_2O_5 as lecithin P_2O_5.	Nitrogen of egg present as—					Nitrogen results in per cent of total nitrogen.			
									Total.	Coagulable.	Uncoagulable.	Proteoses and peptones.	Amido.	Coagulable.	Uncoagulable.	Proteoses and peptones.	Amido.
			Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
AP6	Oct. 17, 1907	Fresh	2.1069	59.3640	0.4097	2.5560	1.7558	68.69	5.0917	4.7054	0.3863	0.1756	0.2107	92.41	7.59	3.45	4.14
AP143	Jan. 17, 1908	do	2.3439	62.0582	.4212	2.6918	1.8769	69.73	4.8160	4.6621	.1531	.0142	.1389	96.81	3.17	.29	2.88
17158	Sept. 7, 1906	Storage 3½ mos	3.0208	61.5535	.3923	2.5177	1.0789	50.00	4.9039	4.6489	.2550	.1255	.1295	94.79	5.21	2.57	2.64
17680B	Jan. 7, 1907	Storage 7½ mos	2.7065	72.5204	.3232	3.8377	.....	.....	5.1707	4.8960	.2747	.1394	.1353	94.69	5.31	2.70	2.61
17919B	June 17, 1907	Storage 12⅜ mos	2.9430	61.9077	.3611	2.6988	1.7345	64.26	4.9885	4.5899	.3987	.2338	.1649	92.01	7.99	4.68	3.31
AP8	Oct. 17, 1907	Storage 16⅝ mos	5.3422	69.0595	.5565	3.7563	2.3372	62.22	6.4413	3.1080	3.3333	3.1830	.1503	48.25	51.76	49.42	2.34
AP147	Jan. 17, 1908	Storage 19¾ mos	3.3754	59.3534	.4473	2.6027	1.6877	64.84	5.1749	1.7588	3.4160	3.3631	.0529	33.99	66.01	64.99	1.02

Analysis of boiled whites of fresh and cold-storage eggs.

WET BASIS.

Serial No.	Date.	Description of sample.	Moisture.	Ash.	Ether extract.	Total sulphur.	Total P_2O_5.	Nitrogen of egg present as—				
								Total.	Coagulable.	Uncoagulable.	Proteoses and peptones.	Amido.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
AP5	Oct. 17, 1907	Fresh eggs	86.15	0.71	0.07	0.21	0.19	1.815	1.51	0.305	0.233	0.072
AP142	Jan. 17, 1908	do	86.08	.55	.16	.28	.....	1.71	1.417	.293	.293	.000
17157	Sept. 7, 1906	Storage 3½ mos	87.41	.95	Trace.	.16	.057	1.79	1.57	.22	.195	.025
17680A	Jan. 7, 1907	Storage 7½ mos	84.24	.82	.....	.19	.350	2.18	1.789	.391	.391	.000
17919A	June 17, 1907	Storage 12⅔ mos	85.02	.76	.....	.219	.098	1.98	1.677	.303	.191	.112
AP7	Oct. 17, 1907	Storage 16⅔ mos	84.53	.76	.14	.21	.20	1.99	1.63	.360	.306	.054
AP146	Jan. 17, 1908	Storage 19⅔ mos	83.14	.48	.25	.29	.13	2.21	1.68	.530	.521	.009

CALCULATED TO A WATER-FREE BASIS.

Serial No.	Date.	Description of sample.	Ash.	Ether extract.	Total sulphur.	Total P_2O_5.	Nitrogen of eggs found as—					Nitrogen results in per cent of total nitrogen.			
							Total.	Coagulable.	Uncoagulable.	Proteoses and peptones.	Amido.	Coagulable	Uncoagulable.	Proteoses and peptones.	Amido.
			Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
AP5	Oct. 17, 1907	Fresh eggs	5.1264	0.5054	1.5162	0.7942	13.1047	10.9025	2.2022	1.6823	0.5199	83.20	16.80	12.84	3.97
AP142	Jan. 17, 1908	do	3.9511	1.1494	2.0115	.....	12.2845	10.1795	2.1049	2.1049	.....	82.86	17.14	17.14	0.00
17157	Sept. 7, 1906	Storage 3½ mos	7.5457	Trace.	1.2708	.4527	14.2176	12.4702	1.7474	1.5487	.1987	87.77	12.23	10.87	1.36
17680A	Jan. 7, 1907	Storage 7½ mos	4.2030	.....	1.2056	2.2801	13.8325	11.3515	2.4810	2.4810	.....	82.07	17.93	17.93	0.00
17919A	June 17, 1907	Storage 12⅔ mos	5.0734	.....	1.4619	.6542	13.2176	11.1950	2.0226	1.2750	.7476	84.68	15.32	9.63	5.69
AP7	Oct. 17, 1907	Storage 16⅔ mos	4.9127	.9050	1.3575	1.9392	12.8636	10.5365	2.3271	1.9780	.3491	81.91	18.09	15.38	2.71
AP146	Jan. 17, 1908	Storage 19⅔ mos	2.8470	1.4858	1.7200	.7711	13.1079	9.9644	3.1435	3.0902	.0534	76.02	23.98	23.56	.41

BACTERIOLOGICAL EXAMINATION.[a]

TECHNIQUE.

Ten fresh eggs and eight cold-storage eggs were examined for the number and the species of organisms present. The technique of this examination was as follows: The eggs were washed in a solution of bichlorid of mercury 1 to 1,000, or 5 per cent carbolic acid, for a few minutes, after which they were dried with sterile absorbent cotton and placed with the large end uppermost in a small beaker. The air space was then scorched with a gas flame for a few seconds. An opening was made immediately into the cavity with sterile forceps, a sufficient amount of shell being removed without rupturing the membrane below. When this was accomplished the latter was broken with a hot platinum spatula and with a sterile pipette 0.5 cc of the white of the egg was quickly removed and placed in the necessary petri plates for cultures. The remaining egg white was then decanted, leaving the unbroken yolk in the shell. With another sterile pipette the yolk sack was ruptured and suitable portions of its contents were removed for study. While such a procedure guards against contamination, the breaking up of the respective layers of the egg when out of the shell is difficult, and sometimes the inability to do so interferes seriously with the obtaining of quantitative results. With the eggs which have been in cold storage for considerable periods a separation of the whites and yolks is not possible.

The first examination of cold-storage eggs, which was made at the expiration of the three months' period, showed white and yolk still separate and the limiting membranes in a fair condition. At the end of six or eight months, however, there was an entire change in the character of the egg, the yolk and white having blended, so that it was necessary to examine the total egg rather than its constituent layers.

DISCUSSION OF RESULTS.

The accompanying tables indicate the results of these examinations. It will be observed that the fresh eggs had originally but few organisms, though in two cases, notably in the yolk, they were fairly numerous. The fact is also noteworthy that the most abundant growth occurred with the fresh eggs at body heat, and later at 15°–20° C. As far as the kinds of organisms are concerned, fungi, yeasts, and bacteria of various types were present. As one would expect, they were mostly air-borne organisms and of the types existing in the soil, cereals, water, etc.

At the expiration of three months in cold storage, as will be seen in the following table, a very great increase in the number of organ-

[a] Made by G. W. Stiles, jr., in charge of bacteriological chemistry, Washington, D. C.

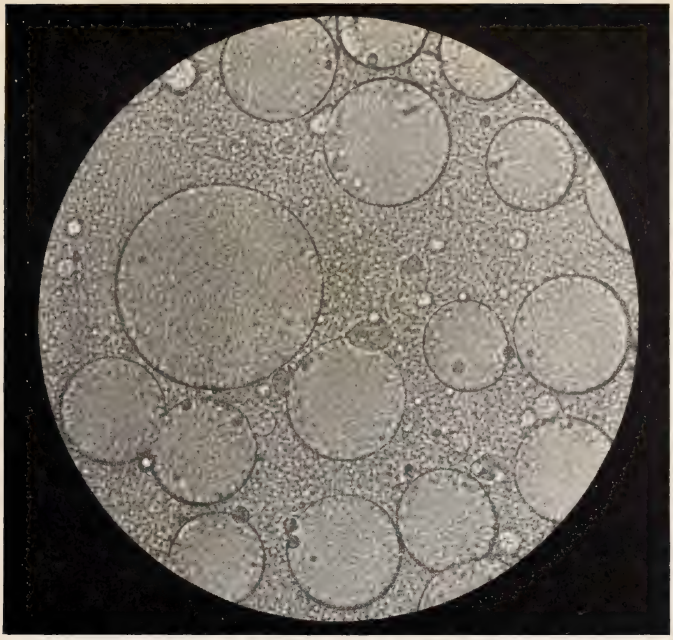

FIG. 1.—YELLOW YOLK OF FRESH EGG (×250).

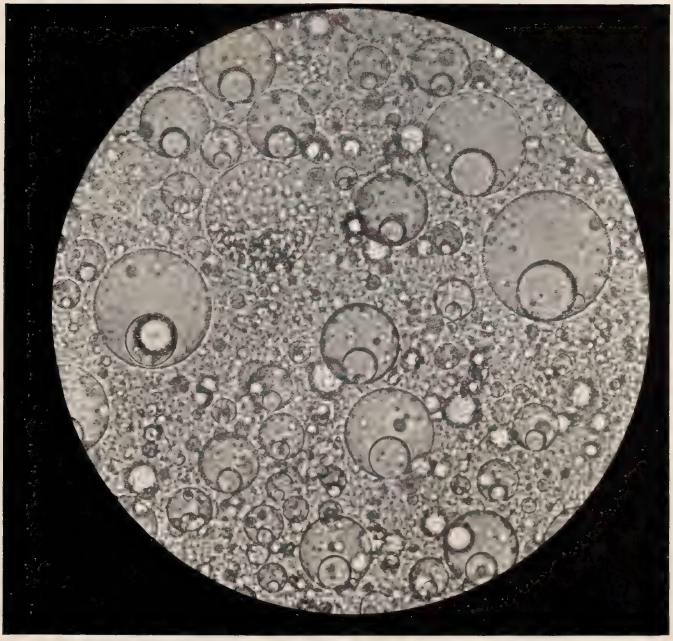

FIG. 2.—WHITE YOLK OF FRESH EGG (×250).

isms was noticed for both white and yolk. The species isolated here are also of a great variety and fairly numerous, as the following list indicates: *Streptothrix* sp.?, *B. vulgatus*, *Sarcina lutea?*, *Alternaria* sp.?, *Sarcina flava*, *Mic. aquatilis*, *Cladosporium* sp.?, *Mic. aerius*, *B. mesentericus*, *Bact. ferrugineum*, *Sarcina subflava*, and *Mic. aureus*.

From this time on until the final examination—which was made when the eggs had been in storage 1 year, 7 months, and 22 days—they were either sterile or inhabited by a few colonies of the more resistant fungi. The number of eggs examined was too small to permit the drawing of elaborate conclusions. The observations are of interest, however, as indicating a line of future investigations.

Bacteriological examination of fresh eggs.

No. of sample.	Received for examination.	Portion of egg.	Number of colonies per cubic centimeter developing on lactose agar at 35° to 37°C.	Number of colonies per cubic centimeter developing on lactose gelatin at 12° to 15°C.	Species of bacteria isolated.
4067	5/24/1906	White	1	0	*Mic. cinnabareus*, Flügge.
		Yolk	190	3	*Myobacterium avium*, *Streptothrix farcinica*, *Sarcina lutea*.
4068	5/24/1906	White	0	0	
		Yolk	0	0	
4069	5/24/1906	White	1	1	*Mic. cumulatus?*
		Yolk	0	0	
4070	5/24/1906	White	1	1	*Bact. haematoides.*
		Yolk	240	3	*Micrococcus*, not classified.
4071	5/24/1906	White	0	0	
		Yolk	5	0	*B. mesentericus.*
4072	5/24/1906	White	0	0	
		Yolk	6	0	*Saccharomyces*, *B. Pammelii.*
B. C. 551	10/17/1907	White	1	0	Fungus.
		Yolk	2	1	1 fungoid colony.
B. C. 552	10/17/1907	White	0	0	
		Yolk	1	0	1 gray fungus.
B. C. 669 *a*	1/16/1908	White	0	0	
		Yolk	1	0	*Actinomyces.*
B. C. 670	1/16/1908	White	0	0	
		Yolk	0	0	

a Gelatin plates incubated at 15° to 20° C.

Bacteriological examination of cold-storage eggs.

No. of sample.	Duration of cold storage.	Portion of egg.	Number of colonies per cubic centimeter developing at room temperatures.
4193	3 mos	White	6,250
		Yolk	3,370
4195	3 mos	White	1,280
		Yolk	3,260
4282	7.5 mos	Total egg	0
4283	7.5 mos	Total egg	1
B. C. 55	11.8 mos	Total egg	1 fungus.
550	11.8 mos	Total egg	White fungus at ice-box temperature.
B. C. 667	1 yr. 7 mos. 22 days	White	1 colony.
		Yolk	1 colony.
B. C. 668	1 yr. 7 mos. 22 days	White	0
		Yolk	0

MICROSCOPICAL EXAMINATION.[a]

FRESH EGGS.

A hen's egg consists of a shell, two shell membranes, the albumen or white of the egg, the yolk, and vitelline membrane. The shell membranes consist of interwoven fibers (Pl. I). Those of the thicker outer membrane are much the coarser (Pl. I, fig. 1).

At the broad end of the egg the shell membranes separate to form an air chamber, which increases in size as the moisture of the egg evaporates.

The albumen, or white of the egg, is fibrillar in structure and consists of a dense portion next to the yolk and an outer, more fluid part. The yolk is formed of alternate concentric layers of yellow and white yolk. The yellow yolk forms the greater part and consists of large spherical granular masses 20 to 109μ in diameter (Pl. II, fig. 1). These masses when hardened in situ are polyhedral, owing to equal pressure (Pl. III, fig. 1). The white yolk is found in a flask-shaped mass in the center of the yolk, and in thin concentric layers which alternate with thicker layers of yellow yolk. The structure differs from the yellow yolk in having spheres of several different kinds which are smaller than those of the yellow yolk 40 to 80μ in diameter (Pl. II, fig. 2).

The vitelline membrane surrounds the yolk and consists of very fine fibrils.

COLD-STORAGE EGGS.

The first eggs examined in the cold-storage experiment were six fresh eggs. These were normal when examined May 24, 1906, and it was stated that the eggs were laid the day before. The next examination was made on a withdrawal of four eggs from storage, September 7, 3.5 months later, and nothing abnormal was noted, the same being true of the withdrawal of January 7, 1907. Another examination was made of the June, 1907, withdrawal, representing a storage period of 12.6 months. At this time two eggs were examined and appeared in general to be normal eggs, but in the yolks were found small rosette crystals. It seemed that they were most frequently found in the outer portion of the yolk. In one egg they were of very rare occurrence. This was the first appearance of these forms and they figured later in the description of the samples of the withdrawal of October, 1907, the last examination made. At this time the entire white of the egg looked watery; the part next to the yolk, although denser than the outer part, had deteriorated. The shell membrane appeared microscopically normal, as well as the vitelline membrane. The yolk assumed a much flattened form after

[a] By Burton J. Howard and E. A. Read, Microchemical Laboratory.

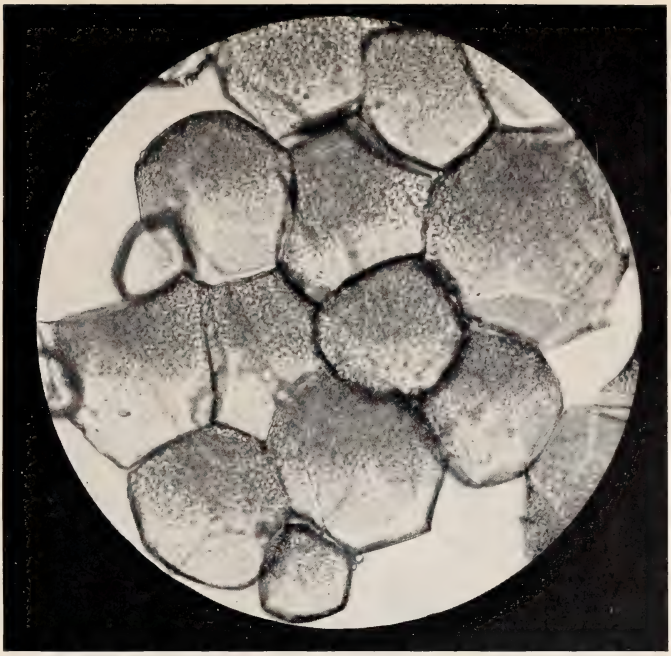

FIG. 1.—YELLOW YOLK OF FRESH EGG, HARDENED IN SITU (×250).

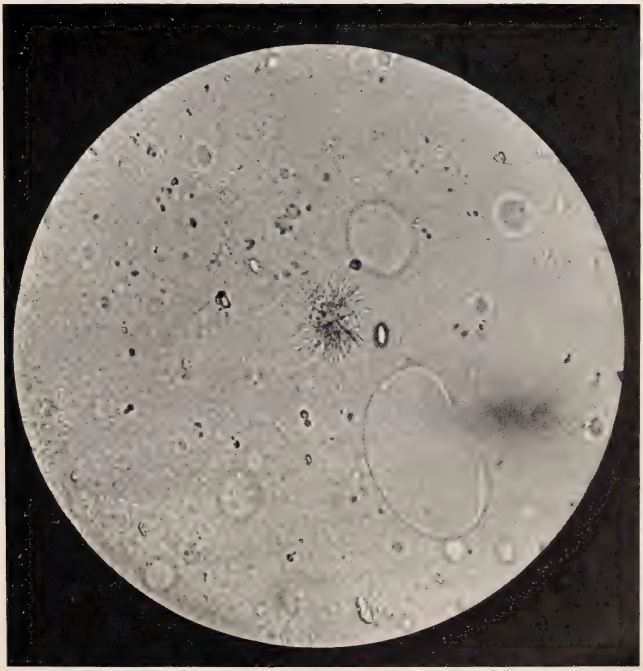

FIG. 2.—ROSETTE CRYSTAL FOUND IN YOLK OF COLD STORAGE EGG (×250).

removal from the shell and did not retain the spherical shape of a fresh egg but in all cases was distinct from the albumen. In addition to the normal yolk elements, rosette crystals were found in the yolk. These measured from 18 to 63μ in diameter (Pl. III, fig. 2). The exact nature of these crystals has not yet been determined, but the following points were established: (1) The crystals are soluble in warm glacial acetic acid but are insoluble in ether, alcohol, or cold mineral acids; (2) the crystals melt and recrystallize when the acids are heated.

SUPPLEMENTARY WORK ON ROSETTE CRYSTALS.

Two eggs were examined about February 15, 1908, when they had been in storage approximately 20 months. In general appearance the eggs were the same as those withdrawn in October, 1907. The rosette crystals were larger and more numerous than those previously found, some of the crystals measuring 109μ.

The study to determine the chemical nature of the crystals is still being carried on, but the difficulties of the problem make the progress very slow. Attempts have been made to separate the crystals for examination, but thus far with only partial success. A portion of the yolk has been mixed on a slide with water and the liquid drawn off, leaving the crystals; the centrifuge methods have been tried, sometimes with large volumes of added water, sometimes without such addition; flooding a slide with alcohol and with ether was also tried as well as combinations of water, alcohol, and ether, but without marked success. Digestion of the yolk with pepsin also proved unsatisfactory. As microchemical methods are usually applied to fairly pure combinations or mixtures instead of in the presence of large amounts of extraneous matter, possibly closely allied chemically with the substance in question, it is seen that this problem is not an easy one, since the crystal forms such an infinitely small proportion of the entire mass of which it is a part.

Certain reactions have, however, been obtained by which it is hoped to establish beyond a doubt whether these crystals are tyrosin, lecithin, etc., but as yet it can only be said that their behavior toward common reagents is very different from that of tyrosin, thus quite definitely indicating that they are not tyrosin crystals.

II. QUAIL COLD-STORED UNDER KNOWN CONDITIONS.

ORGANOLEPTIC TESTS.

The quail were placed in cold storage on March 1, 1906, and examined before and after cooking at periods varying from 3 to 5 months, making three cold-storage tests during the year. The fresh quail sampled on March 2, 1906, were voted sweet and fresh, of good flavor, but somewhat dry. The birds were cooked by the same person throughout the tests and prepared as nearly the same as possible.

Some variations are necessarily introduced by the original differences in the quality of the birds, the extent to which they were shot, etc. Nevertheless, the table shows a practically uniform vote.

After 3 months' storage no deterioration was noted, and no distinction could be made between the drawn and the undrawn bird. The flavor was still sweet, though the birds were perhaps somewhat drier. After 8½ months in cold storage, the fresh bird was distinguished from the others without difficulty by all of the jurors, and, in practically all of the cases, the undrawn birds were declared to be already marked by stronger taste and odor. It is, of course, understood that these birds were designated by numbers, and the jurors recorded their opinions independently and without any knowledge of the identity of the birds.

After about 1 year in storage, the difference between the fresh and cold-storage birds was again marked, though the drawn cold-storage bird is rated uniformly as second best, and is tasteless rather than strong; while the undrawn birds were recorded as bad or strong as to either taste or odor in practically every case, though the larger undrawn bird is recorded in two cases as superior to the small one and of good flavor. There can be no doubt but that after 8 months of storage the birds were markedly inferior to the fresh ones, when simply cooked and seasoned, and in nearly all cases the deterioration of the undrawn bird is much more marked than that of the drawn one, in so far as the odor and flavor can testify.

In addition to the table tests, the birds were examined before cooking, with the following results: On June 5, 1906, at the time of the first cold-storage test, the undrawn bird appeared better than the drawn bird in every way, being plumper, lighter colored, firmer, and of better odor.

At the time of the test on November 15, the appearance and odor of the undrawn quail was still superior to that of the drawn birds, the odor of the drawn quail being more rancid and comparing less favorably with the fresh quail than those that were undrawn.

At the inspection on February 19, 1907, when the birds had been in storage nearly a year, two of the examinations show that the appearance, especially the inside of the undrawn bird, was very bad. The drawn bird, however, had a stronger odor, and there does not seem to be much difference between the two as a whole, both being somewhat dry and shriveled. The results may be compared with the bacteriological findings given in the following report, the birds stored for corresponding periods being from the same lots. It is evident that the contention that the undrawn bird presents a better appearance is true, but that it is inferior in flavor and odor when served is also true, and it remains for the chemical, bacteriological, and histological examinations to show whether more serious deterioration has taken place in the case of the undrawn bird.

Quail test.[a]

JUNE 5, 1906 (3 MONTHS IN STORAGE).

No. of bird.	Key.	Juror. H. W. W.	A. L. P.	M. T. R.	F. P. V.	T. C. T.	F. C. C.
1	Drawn	Taste and odor good; no deterioration.	Good, but dry; no deterioration; more gamy.	Fairly firm, sweet, good flavor.	Good	Good	
2	Undrawn	Taste and odor good; about like No. 1.	About like No. 1; less flavor.	Not so palatable as No. 1; white meat dry; dark, firm, and sweet.	Somewhat sweeter than No. 1; both drier than fresh birds.	Good; dark meat more gamy than No. 1.	

NOVEMBER 15, 1906 (8½ MONTHS IN STORAGE).

1	Drawn—worst appearance.		Taste and odor sweet; dry.	Taste and odor good.		Poor and rancid; odor not good.	Taste dry; odor good.
2	Undrawn—worst appearance.		Odor not as sweet as No. 1; taste much less sweet.	Taste strong; odor good.		Dry and no flavor; very poor.	Flavor poor; odor good.
3	Drawn—best appearance.		Dry; tasteless; inferior to Nos. 1 and 2.	Taste and odor strong.		Dry; odor bad	Taste poor; odor good.
4	Undrawn—best appearance.		Dry; tasteless	Taste strong; odor strong and disagreeable.		Bad	Taste poor; odor fair.
5	Fresh		Taste and odor sweet; more flavor than other birds; fresh.	Taste sweet; odor good.			Flavor and odor good.

FEBRUARY 19, 1907 (11½ MONTHS IN STORAGE).

1	Drawn	White meat tasteless and tough; meat around joint of leg rancid.[b]	Odor sweet; white meat dry; dark meat rather strong.	Tasteless and tough; odor good.	Second best in taste; odor not bad.		Appearance and odor good; fat a little rancid.
2	Fresh	Taste better than No. 1; odor good.	About like No. 1; superior to No. 1 in taste.	Taste better than No. 1; odor good.	Taste and odor fresh		Appearance and odor good; taste better and sweeter than Nos. 1 and 3.
3	Undrawn	Tasteless; odor bad	Large bird, fairly good, inferior to No. 2; smaller one, taste strong, odor offensive.	Taste and odor strong.	Small bird, taste and odor very bad; large bird, taste and odor very good.		Poor flavor; odor rather disagreeable.

[a] Placed in storage March 2, 1906.

[b] M. E. P. substituted for H. W. W.

BACTERIOLOGICAL EXAMINATION.[a]

The bacteriological examination of quail was conducted along the same lines as was the examination of cold-storage and fresh chickens. As before stated, they were killed especially for this work. Half of the number was drawn, the other half left undrawn, and all placed in the freezer as promptly as possible. The feathers were not removed. Thawing was ordinarily allowed to proceed at 10° to 12° C., after which the examination was conducted as promptly as possible.

Because of the difficulty in obtaining fresh birds at all seasons of the year, it was sometimes necessary to examine those in storage without having a fresh bird at the same time for comparison. Whenever possible, however, fresh birds were obtained, and in one case a bird which had been 3 weeks in storage was used as a basis of comparison. Even so short a period, however, caused minor irregularities, hence the findings for this quail can not be accepted as absolute.

In all, 4 fresh quail have been examined, 3 being from the same lot. No. 4272 was examined as the comparate of the birds in storage for 11.5 months. The other fresh quail were tested simultaneously with the birds in storage for 13 months.

No. 4272 showed a number of bloody spots, which indicated the site of the shot wounds. It had also a broken leg and a broken wing. The body cavity showed that the shot had penetrated and had torn the viscera as well as broken the intestinal walls, which permitted the diffusion of the contents throughout the body cavity. Such a condition would of course infect any organ which had been damaged by shot. Those which were not so damaged were sterile. The species isolated, and their location, are listed in the table under sample No. 4272.

Nos. 752 and 754 were in good condition except for shot wounds which had torn the viscera of No. 752. No. 753 was badly torn, both externally and internally, and was dark in color for a fresh bird. There was no marked odor or discoloration, yet decomposition had apparently begun.

The first examination was made when the quail had been in storage for three months. The external appearance of both the drawn and the undrawn quail was good. The body, however, was altered in shape from pressure while in the case. The undrawn was the better looking of the two. Both birds had a peculiar odor, not very strong but suggestive of rancid fat, which was more pronounced in the undrawn than in the drawn. Several small areas on the breast muscle showed evidences of desiccation, and in the drawn quail this muscle showed shot wounds, which were surrounded by a bloody exudate. The fibers of the muscle were brittle.

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[a] Examination conducted by G. W. Stiles.

The viscera of the undrawn bird were in fairly good condition. A small amount of a dark extravasated bloody effusion was found in the abdominal portion of the body. There were no gas bubbles and no discoloration except where the liver came in contact with the parietal walls.

The body cavity of the drawn bird contained portions of the liver and kidneys. These were slightly softened and dark in color. The blood which was in the body cavity was quite dry. No particular discoloration of the tissues was noted and the bones appeared normal. As will be observed in the table under No. 4073, this quail had an infected brain, and also gave a bacterial growth from the breast muscle in the region of the shot wound. This muscle, however, near the crop where the skin was unbroken, was sterile. The only organism isolated in pure culture was *Micrococcus tenacatis*.

In the undrawn quail bacteria were found in the leg muscle. The leg, however, had been broken; hence it is possible that the infection was local. The organisms isolated are given in the table.

The second examination of quail preserved by cold was made after a storage period of 8.5 months. In both birds the odor was suggestive of rancid fat. In the undrawn bird, however, there was a distinct fecal odor in addition. The region of the vent in both birds was darkened in color, with rather a greenish tinge in the undrawn. The muscles of both birds cut very readily.

The abdominal cavity of the undrawn bird contained about 10 cc of a bloody serous fluid. The kidneys were dark and congested; the intestines of a dull gray color not at all attractive in appearance. There was also a noticeable odor of putrefaction when the abdominal cavity was first opened. The membranous lining of this cavity had a slimy appearance and a greenish-yellow tinge.

In the drawn bird the thoracic portion of the cavity was in very good condition, but the posterior region was abnormal both in appearance and in odor. The lining membrane, particularly in the region of the vent, was infiltrated with a slimy, bloody fluid.

The crops in both birds contained the seeds composing the food of the quail, and a few gas bubbles were found entangled within the loose connective tissues surrounding it. The muscle was apparently in good condition, the only discoloration being where the shot had penetrated. The only organisms isolated from these birds were from the intestines, cultures having been made from various regions as indicated in the table, where the quail are numbered 4270 and 4271, respectively.

The next examination was made when the quail had been in storage for 11.5 months. The general external appearance of both the drawn and the undrawn birds was bad. The body was distorted in form and broken bones were noticed in both cases. The muscles

were shriveled and the region of the vent was distinctly sunken, varying in color from green to a brown-black, the discoloration extending well into the tissues of the inner side of the leg. The skin was parchment-like. All of these conditions were more pronounced in the undrawn than in the drawn quail. The odor was putrefactive, rancid, and fecal for the undrawn, and distinctly rancid for the drawn bird.

The muscles of the drawn bird were in better condition than were those of the undrawn. The texture of the latter was flabby and without elasticity.

In the undrawn bird the abdominal cavity showed the presence of mites. The heart was in fair condition. All the other organs were in exceedingly bad condition, the liver, for instance, being soft, light green or yellow, and much degenerated. The kidneys were dry and friable, varying from gray to yellow-brown. The intestines were green, the folds being matted together in masses without any distinctive outlines of demarcation, and the walls so thin that they broke even when lightly touched. This condition grew worse toward the vent.

In the drawn bird the body cavity contained blood which was nearly black, the walls of the cavity being greenish-yellow near the vent. The lungs and kidneys were in situ, but so soft and darkened in color that their structure was almost lost. Cultures from these birds were made and the results are given in the table under No. 4431 and No. 4432. All the cultures made from the drawn bird were sterile. From the undrawn bird growth was obtained in a number of instances, and the species isolated were varied both in character and in location.

The bird used for comparison (No. 4433) had been kept in storage for three weeks, this period being necessary because of the closing of the season for the killing of quail. The bird was in a very good state of preservation. However, the region of the vent showed a slight greenish-yellow tinge. There was a shot wound in the right leg, which still showed the shot embedded in the tissue, and the inner thigh muscles were discolored and hemorrhagic, this condition extending well toward the vent. The lungs were somewhat congested and the kidneys slightly darker than those of the fresh birds before examined. All the cultures made from this bird proved to be sterile.

After a storage period of 13 months, the two undrawn birds—Nos. 755 and 756—had discolored skins, especially in the region of the vent. The muscles were also discolored and dry. The viscera were in bad condition, dark in color, the intestine leaden, the tissues degenerated even to macroscopic observation, and a bloody, slimy fluid present in the body cavity. The odor was fecal.

The drawn birds of this lot—Nos. 757 and 758—were in a bad state of preservation externally and internally. The breast muscles were soft and degenerated, stained red where in contact with the bone, and the bone marrow was absorbed. The portions of the viscera left in situ were dark, bloody, gaseous, and slimy.

It will be observed from even the small number of birds examined, and here listed, that the problem becomes even more complicated than in the case of the chickens, because the method of killing is almost certain to introduce bacteria which may be carried into the deepest tissues and, as in the case here noted, result in the rupturing of the intestine and the pollution of the entire body cavity. The prompt dressing and cooking of birds so injured would render this factor unimportant. If, on the other hand, the birds are exposed for sale for a number of days before being disposed of, or being unsold are, at the expiration of such a time, transferred to cold storage, the condition may be menacing. It would seem, in the case of quail even more than in the case of chickens, most desirable that a very prompt transference to the storage warehouse be made, or that the birds be dressed and sterilized by heat as soon as possible after killing.

Bacteriological examination of fresh and cold-storage quail.

FRESH QUAIL.

No.	Description.	Time of storage.	Cultures. From—	Cultures. Result.	Species isolated.
		Months.			
4272	Undrawn		Heart blood	0	
			Liver	0	
			Lungs	+	*Strep. pyogenes?, Aspergillus glaucus*
			Kidney	+	*Staph. epidermis?*
			Breast muscle near crop	0	
			Peritoneal fluid	+	*Strep. pyogenes, Mucor racemosus.*
752[a]	Undrawn		Liver	+	*Bact. salmoneum?*
			Kidney	0	
			Breast muscle near crop	0	
			Leg muscle	0	
			Bone marrow	0	
753[a]	Undrawn		Heart blood	+	Culture 737.[b]
			Kidney	+	
			Breast muscle near crop	+	Culture 737.[b]
			Leg muscle	+	*B. subtilis* group.
754[a]	Undrawn		Heart blood	+	Culture 737.[b]
			Liver	+	*M. pyogenes albus.*
			Kidney	+	*M. pyogenes albus.*
			Breast muscle near crop	0	
			Leg muscle	0	
			Bone marrow	0	
			Peritoneal fluid	0	

STORAGE QUAIL.

4433	Undrawn	¾	Heart blood	0	
			Liver	0	
			Kidney	0	
			Breast muscle near crop	0	
			Peritoneal fluid	0	
4073	Drawn	3	Brain	+	
			Heart blood	0	*M. tenacatis.*
			Breast muscle near crop	0	*M. tenacatis.*
			Breast muscle near wounds	+	*M. tenacatis.*

[a] From same lot of quail, results comparable.
[b] All cultures numbered refer to bacteria not classified.

Bacteriological examination of fresh and cold-storage quail—Continued.

STORAGE QUAIL—Continued.

No.	Description.	Time of storage.	Cultures. From—	Cultures. Result.	Species isolated.
		Months.			
4074	Undrawn (same lot as 4073).	3	Brain	0	
			Heart blood	0	
			Breast muscle near crop	0	
			Leg muscle	+	*B. cloacæ, Spir. serpens.*
4271	Drawn	8.5	Brain	0	
			Lungs	0	
			Breast muscle near crop	0	
			Bone marrow	0	
4270	Undrawn (same lot as 4271).	do	Brain	0	
			Heart blood	0	
			Liver	0	
			Kidney	0	
			Intestine	+	*B. circulans?, M. aurantiacus.*
			Breast muscle near crop	0	
			Bone marrow	0	
			Peritoneal fluid	0	
4431	Drawn	11.5	Heart blood	0	
			Breast muscle near crop	0	
			Breast muscle near wounds.	0	
4432	Undrawn (same lot as 4431).	do	Heart blood	+	*Strep. erysipelatos.*
			Liver	+	*M. tenacatis?*
			Lungs	+	*B. enteritidis.*
			Intestine	+	*M. tenacatis?, B. capillaceus.*
			Pericardial fluid	+	*Strep. erysipelatos.*
			Breast muscle near crop	0	
			Peritoneal fluid	0	
757	Drawn	13	Breast muscle near crop	0	
			Leg muscle	0	
			Bone marrow	0	
758	Drawn (same lot as 757).	do	Breast muscle near crop	0	
			Leg muscle	0	
			Bone marrow	0	
755	Undrawn (same lot as 757).	do	Heart blood	+	Feeble growth, failed to develop when transplanted.
			Liver	0	
			Intestine	0	
			Breast muscle near crop	0	
			Leg muscle	0	
			Bone marrow	0	
756	Undrawn (same lot as 757).	do	Heart blood	0	
			Liver	0	
			Kidney	+	Feeble growth, failed to develop when transplanted.
			Intestine	0	
			Breast muscle near crop	0	
			Leg muscle	0	
			Bone marrow	0	

III. CHICKENS COLD-STORED UNDER KNOWN CONDITIONS.

ORGANOLEPTIC TESTS.

Tests were made on chickens placed in storage on July 1, 1906, and withdrawn at intervals of approximately three months for a period of almost nineteen months. At each test a drawn and an undrawn chicken were taken out of cold storage and compared with a fresh chicken both before and after cooking, the jurors having no information as to the history of the birds and giving their opinions independently. The chickens were cooked by the same person throughout the experiment and prepared in the same manner.

On October 19, 1906, after three and a half months of storage, the cooked chickens showed practically no deterioration and the jurors found it a little difficult to distinguish them from the fresh fowl.

At the second test, however, on January 16, 1907, three months later, the fresh bird was easily distinguished by all of the jurors. This was also true in the other three tests, the difference becoming more pronounced at each trial, and only one mistake was made during the entire observation in the distinction between the fresh and the cold-storage fowls. About 70 per cent of the votes cast at the second test declared the undrawn fowl to be inferior because of its darker color and its stronger odor and taste.

After almost a year of cold storage, June 11, 1907, the third test was made. The undrawn cold storage fowl was correctly designated by all except one of the jurors, and was described as being of dark color with a bad odor and strong taste.

Four months later, in the test of October 22, 1907, when the birds had been in storage for 15.5 months, the distinction between the drawn and undrawn cold-storage birds was less pronounced, but there was no question as to which of the three birds was fresh. About 70 per cent of the votes again indicated correctly the undrawn birds, which were described as being dark and dry, the bone at the joints darkened, the white meat tasteless, and the dark meat strong in the majority of cases.

When the last test was made, on January 23, 1908, the birds had been in cold storage for 18.5 months. Although the difference between the fresh and the storage birds was obvious, the jurors found it even more difficult than in the preceding test to discern the difference between the drawn and the undrawn storage chicken, the vote being evenly divided; both of the storage fowls were by this time somewhat dry and dark, and tasteless rather than strong.

From the evidence of the jurors who examined the cooked birds, the following conclusions are drawn: The undrawn fowl is distinguished at first with comparative ease, being inferior in appearance and usually stronger in odor and taste. When they have been stored for 18 months, however, it is difficult to discern a difference in the two, as both are dry and tasteless. The jurors are practically never in doubt as to the superiority of the fresh bird, and this becomes more marked as the experiment progresses, though a short period of storage (3 to 6 months) seems to cause no deterioration.

Besides the table tests, the fowls were subjected to a macroscopic examination before being cooked. When the chickens had been in storage for 6.5 months, the verdict was that both externally and internally the undrawn bird was better in odor and appearance than the drawn cold-storage bird.

On June 11, 1907, the second examination was made after a storage period of nearly a year, and it was found that the internal appearance of the drawn fowl was still good, no serious decay having apparently occurred. The internal examination of the undrawn chicken

showed signs of degeneration of the organs, but the odor was not bad, though a little stale. Both fowls were in fairly good condition.

After 15.5 months of cold storage, on October 22, 1907, the undrawn bird again presented a better appearance generally than the drawn one, both internally and externally. The flesh of the two fowls was somewhat discolored, but the odor was not bad. The liver, the lungs, and the muscle tissue were all in better condition in the undrawn chicken.

On January 23, 1908, when the chickens had been in cold storage for 18.5 months, they were again examined. There was practically no disagreement as to the inferiority of the undrawn bird in this test, both the internal and external appearance, and also the odor, showing clear evidences of degeneration.

The conclusion drawn from the examination of the uncooked chicken is that after 12 or 15 months of cold storage the undrawn chicken appears better than the drawn, while after 18 months in storage the undrawn chicken is in decidedly the worse condition of the two in every respect.

Detailed descriptions of the appearance of the chickens at the last two examinations are appended and the results obtained in the organoleptic tests are comparable with the bacteriological findings of corresponding dates, such birds having been from the same lot.

Macroscopical examination of fresh and cold-storage chickens, October 22, 1907, after 15 months of cold storage.

F. C. W.

Fresh (black chicken).—Color of skin white; tissues normal, soft and elastic with normal odor; breast muscles soft, yielding, and elastic, nearly colorless; thigh muscles normal, the various muscles being easily differentiated by color, muscle sheath, and elasticity. Liver firm, elastic, and of normal color; gall bladder normal; intestines firm, resistant, contour well marked, normal color, small blood vessels noticeable.

Drawn.—Yellow color; odor not bad (too cold to test); tissues dry, firm, dehydrated, and adherent in places, though not to such an extent as the undrawn bird; breast muscles dry, moderately firm, striations not so marked, lighter pink color and better appearance than undrawn chicken; thigh muscles hard, dead appearance; colors of various muscles not differentiated. Liver and lungs hardly recognized from their gross appearance; heart not a bad color; gizzard fairly good condition.

Undrawn (Barred Plymouth Rock chicken).—External color yellow; odor not bad (bird too cold to test); tissue dry, firm, dehydrated and adherent in places; breast muscles moderately dry, firm, striations rather marked, dull pink color nearly red, not natural; thigh muscles hard and rather dry near external surface, dark red in color; muscles not clearly differentiated in color and muscle sheaths not well marked. Liver soft, faded and mottled color, with tissue neither natural nor elastic; gall bladder partly empty and pale green; gizzard a little darker than normal; heart not a marked blood color; intestines soft, mushy, and pale, all traces of blood vessels gone; fat rather yellow.

M. E. P.

Fresh.—Eyes full, comb and gills pale; neck soft and well rounded; feather papillæ prominent. Region of crop inconspicuous, but not depressed. Body clear pale pinkish yellow color, with bluish tones over legs and hips; skin firmly but pliably attached to tissues beneath. Muscles of breast almost colorless, opalescent grayish pink; texture very elastic. Muscles on inside of thigh pink and soft; fat pale yellow. Dark, blood-red liver, well rounded intestines, blood supply distinct; gizzard bright.

Drawn.—Head gone; end of neck powdery, dry; skin like parchment, adherent to neck, loose over breast, easily torn away from muscles, much too yellow; tissues below dried out. Muscles of breast too pink and dried, fibers very prominent, elasticity largely gone. Inside of thigh too deep in color, purplish and brownish tints appearing; fat too yellow. Gizzard, liver, and lungs loose in body cavity, hardly recognizable.

Undrawn.—Eye depressed, comb and gills dried; neck shrunken, skin adherent. Skin better than drawn, but not normal. Breast muscles too pink, very fibrous, not elastic, not so dry as drawn and generally in better condition. Tissue of thigh better than drawn, but tending toward some coloring. Liver lighter in color and not resistant to pressure, mottled; gizzard normal; intestines flattened, veinings indistinct; color not bad. Gall bladder has discolored tissues around it. Fat slightly discolored.

F. C. C.

Fresh.—Skin light in color and tight; odor and appearance good; interior good norm a color, with organs fresh; odor as in all fresh chickens.

Drawn.—Skin rather dry and hard; odor and appearance good. Internal organs discolored and shrunk, but not of bad odor; muscle fibers dried and striations very distinct; odor all right.

Undrawn.—Skin dry and hard; odor and appearance good; appearance of interior better than in drawn fowl; the organs fairly normal, only slightly discolored. Muscle tissues look better than in drawn chicken.

Macroscopical examination of fresh and cold-storage chickens, January 23, 1908, after 18 months' cold storage.

H. W. W.

Fresh.—Yellow and pink flesh; purple and red legs; plump; pleasant odor; eye full; interior flesh and intestines red; odor fresh; liver brownish red; lung tissue yellow.

Drawn.—Faded color; flint legs; shriveled skin; bad odor; head gone; interior flesh appears more nearly normal than that of undrawn; odor also better.

Undrawn.—Faded color, pale, and shriveled; bad odor; eye sunken and almost invisible; red color of intestines gone; liver bright yellow, very bad odor; interior flesh faded and bad smelling; many evidences of degeneration.

F. C. W.

Fresh.—Color normal pinkish; odor as of fresh meat; tissues soft, friable, and moist; eyes normal.

Drawn.—Tissues dry, hard; color darker than normal; skin drawn and tightly adherent; odor better than undrawn; head off; interior tissues tough with no resilience; odor and color better than undrawn.

Undrawn.—Muscles dry and hard with skin tightly drawn and adherent; color dead and dark; odor stale; eyes sunken; interior tissues pale, hard, and tough, with no resilience, bad fecal odor; lungs mottled white; liver flabby; gall bladder half empty; gizzard smaller than normal; intestines soft, mushy, with no blood in the blood vessels and through omentum; blood vessels in walls have disappeared.

F. C. C.

Fresh.—Rather small chicken, 1½ pounds; color and odor normal; eye full and round; interior color and odor normal; liver brown-red.

Drawn.—Skin and muscles rather bruised; skin dry; not plump; odor good; interior color pale, but more normal than undrawn, and odor good, though not strictly fresh.

Undrawn.—Skin dry and bruised; shape lost owing to packing; eye has disappeared; odor good; interior color very pale; meat has lost its characteristic appearance; hemoglobin reduced.

Taste, odor, and condition of cold-storage chickens, cooked and tested at intervals of three months. Period of storage, July 1, 1906, to January 23, 1908.

OCTOBER 19, 1906 (3½ MONTHS' COLD STORAGE).

Key.	M. T. R.	F. P. V.	T. C. T.	H. W. W.	F. C. C.	A. L. P.
No. 1—drawn	Normal; meat firm; odor and taste sweet.	Drawn; taste good; odor a little stale; not very attractive.	Appearance and odor good; taste dry.	Drawn; appearance medium; odor fair; taste medium.	Good appearance, taste, and odor.	
No. 2—undrawn	Same	Appearance worst of three; taste flat; odor good.	Appearance not as good as No. 1; odor same; very little taste.	Appearance poorest; odor poor; taste poor.	Good taste; best of three.	
No. 3—fresh	Same	Appearance like No. 1; odor good; sweetest of three.	Appearance very poor; odor same as No. 1; taste not as dry.	Appearance best; odor fair; taste medium.	Appearance inferior; odor and taste good.	

JANUARY 16, 1907 (6½ MONTHS' COLD STORAGE).

No. 1—fresh	Appearance attractive; odor and taste good; dark meat tough.	Juicy; good odor and taste; best.	Appearance, odor, and taste good; a little tough.	Taste, odor, and appance good; rather tough.	Appearance good; odor good; fresh and palatable.	First class; odor very sweet; flavor fair; rather tough.
No. 2—drawn	Not so white or solid as No. 1; odor not so good; strong, could not eat it.	Second best; tasteless; odor not as good as No. 1.	Appearance dark, poor; odor negative; taste rank, could not eat it.	Small and shriveled; odor bad; tasteless, but not bad.	Appearance good; odor not as good as No. 1; slightly dry; left unpleasant aftertaste.	Appearance not so good as No. 1; odor negative; more tender than No. 1; flavor fair.
No. 3—undrawn	About like No. 2; stronger odor, but taste not as bad.	Worst; odor not as good as No. 2; taste better than No. 2.	Appearance dark, but better than No. 2; odor bad; taste of dark meat bad, white meat dry and poor.	Color bad; bluish on back; odor very offensive; taste bad.	Appearance good; odor not good, contamination from intestinal tract; unpalatable, strong aftertaste.	Shriveled, dull in color; odor strong; taste, especially of dark meat, very strong.

JUNE 11, 1907 (STORAGE FOR ABOUT 11 MONTHS AND 1 WEEK).

No. 1—fresh	White meat good and firm; odor poor; white meat tastes good; dark meat flabby.	Appearance, odor, and taste fresh and good.	Good chicken; appearance, taste, and odor fresh and good.	Appearance fair; odor and taste good.	Appearance, odor, and taste fresh and good.	Appearance fair; tastes better than it smells; tender and sweet; probably drawn storage fowl.
No. 2—drawn	Appearance not as good as No. 1; odor better than No. 1; taste good.	Not as good as No. 3; bone dark; odor stale; taste flat, bad next to bone.	Dark, not good; tastes very bad; poor generally.	Appearance: External fair, interior indifferent; odor bad; tasteless or of rather bad flavor.	Appearance and odor better than No. 3; white meat bad aftertaste, not fresh.	Odor fresh and most excellent appearance; not as good as No. 1; rather coarse and tough.
No. 3—undrawn	Appearance good, meat not as firm as No. 2; odor strong; taste so bad could not eat it.	Appearance fairly good; better than No. 2; odor stale, better than No. 2, as is also taste.	Too bad to eat; worst of three; looks dark.	Appearance: External fair, interior indifferent; odor bad; taste very bad; worst of three.	Not as fresh as No. 2; odor slightly bad; taste not good, but better than No. 2.	Strongest of the three; white meat strong, tough, and dry.

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Taste, odor, and condition of cold-storage chickens, cooked and tested at intervals of three months. Period of storage, July 1, 1906, to January 23, 1908—Continued.

OCTOBER 22, 1907 (15½ MONTHS).

Key.	M. T. R.	F. P. V.	T. C. T.	H. W. W.	F. C. C.	A. L. P.
No. 1—fresh	No. 1 best[a]	Appearance normal; odor and taste good and fresh.	Looks, smells, and tastes very good; fresh chicken.	No. 1 best chicken; color, taste, and odor good.	Bones white; fresh odor; taste very sweet and good; fresh chicken.	Appearance and taste good; bone white; odor sweet; best chicken.
No. 2—drawn	No. 2 better than No. 3.	Bone darkened; worst appearance and odor of three; rather tasteless.	Rather dry and brown; very little odor or taste; second best.	Somewhat strong odor; white meat slightly yellowish and inferior to No. 1 in taste; dark meat very bad taste and smell; voted undrawn fowl.	Bones and white meat dark at joints; smell of fat not fresh; dry; not as palatable as No. 1; second best.	Stronger and dryer than No. 1; voted drawn.
No. 3—undrawn	No. 3 worst	Bone darkened, not so yellow as No. 2; odor stale; more taste than No. 2; voted second best.	Looks very dry and dark; very little odor; tasteless.	White meat dry; dark meat strong in odor and taste; voted as drawn fowl.	Bones deteriorated at joints; odor not fresh; white meat dry and fat slightly rancid; poorest of three.	Very dry; taste of dark meat bad; bone dark; worst of three; voted undrawn.

[a] M. E. P., substitute juror.

JANUARY 23, 1908 (18½ MONTHS).

No. 1—fresh			Looks, tastes and smells very good; best chicken.	Flesh natural; odor fairly good; taste good; best of the lot.	Fresh; bones white at joints; odor and taste good.	Odor strong; dark and dry; taste moist, not strong as expected from odor and appearance; more flavor than No. 2; best.
No. 2—drawn			Appearance and odor all right; very dry and but little taste; second best.	Strong odor; taste of white meat fairly good; unnatural appearance of cut surface of flesh; worst.	Taste not so good as No. 1; bones dark at joints; odor not fresh; second best chicken.	Bone dark; very little odor; dry and tasteless, differing little from No. 3.
No. 3—undrawn			Appearance dark and dry; but little odor; taste dry and tough; poorest of the three.	Flesh not natural; odor bad; tasteless; medium.	Odor not as fresh as No. 1; bones dark at joints; taste worst of three.	Smaller bird than No. 2; tastes sweet, but tough; white meat dry, dark meat a little strong; second best.

BACTERIOLOGICAL EXAMINATION.[a]

The bacteriological study of the fresh and cold-storage fowls has been concerned with the presence of bacteria in the tissues, and their species. The birds were sent to the bacteriological laboratory from a lot obtained for general work and, previous to their reception at least, were in nowise differently treated from the others. When received at the laboratory they were examined immediately, or, if this was impossible, they were placed on ice for the short period of waiting.

The portions of muscle to be examined were obtained by searing the surface to insure sterility; then small pieces were removed with a stiff platinum hook such as is commonly used for this purpose. The fragments were transferred to suitable media and incubated at body heat, and in many cases also at from 15° to 20° C. The media used in routine consisted of plain agar, nutrient bouillon, plain gelatin, glycerin agar, and, for the detection of gas-forming organisms, a 2 per cent dextrose broth. Growth from the fresh chickens occurred with a fair degree of promptness. In the cold-storage chickens, on the other hand, six days or more were required at 20° C.

A close examination was made of the physical condition of the birds, and brief descriptions of them are here given. The results obtained with the fresh chickens are tabulated in the table. These fowls were all in good physical condition. They were drawn, and had been picked. The muscles were elastic, yet tender. The birds were killed by puncturing the spinal cord by way of the mouth. Hence there was a wound in the head, and, owing to the manner of killing, it was of course more or less infected with organisms from the knife. Here and there there were slight abrasions of the skin and sometimes the wing bones were broken, such accidents being likely to happen in the killing and picking of the fowls. Internally they were all in good condition. Parasitism was not noticed in any case. The odor of the flesh was pleasant, lacking rancidity. The tissues in the region of the gall bladder had rather a greenish tinge.

Two of the six fresh chickens examined were sterile, cultures being made from heart blood, liver, spleen, bone marrow, and muscle tissue. All of the others showed one or more organs infected. The variety of organisms isolated, however, was not large. In this connection it should be noted that chicken No. B. C. 559, received shortly after killing, was kept in a sterile metal container on ice overnight. It was, therefore, somewhat more than twenty-four hours old when the examination was made. No. B. C. 694 was kept on ice under the same conditions for a few hours only.

The first examination of cold-storage fowls was made when they

[a] Conducted by G. W. Stiles, jr.

had been preserved under the conditions previously noted for 119 days. An undrawn and a drawn specimen were examined simultaneously, and comparisons not only with a fresh fowl but with one another were made.

From the undrawn fowl only the feathers were removed. From the drawn, the head, feet, legs, crop, and intestines were removed. Originally the lungs, heart, liver, spleen, testicles, and kidneys were left in position, and the gizzard was emptied and returned to the body cavity.

Externally both of these chickens showed some broken skin. Both had an odor, but that of the undrawn fowl was faint and not unpleasant, though rather different from that of the fresh bird, but distinctly better than that of the drawn fowl, where indications of beginning putrefaction were plainly evident—a condition observed for the undrawn around the head only. That portion, however, was distinctly foul. In the undrawn chicken there was no apparent change in the color, while in the drawn there was a tinge of greenish yellow in fat and subcutaneous muscle.

The viscera of the undrawn fowl were in very good condition, and the fecal odor noticed when the incision was first made soon disappeared. There was some bloody effusion present in the lymph spaces of the abdominal cavity. This was always noted for the drawn bird. The liver was pale in color, but of good texture. It was somewhat green where in contact with the gall-bladder ducts. The testicles and mesenteric fat were somewhat more yellow than in the fresh chicken. This also applied to the chicken from which the viscera had been removed. Cultures were made both from the organs and from the muscles.

The infected organs and also the list of species, and their sources, in the chicken will be found in the accompanying table under specimens Nos. 4251 and 4252, respectively.

The second examination of cold-storage chickens was made when storage had continued for 215 days. As in the former examination, there was a comparison of the drawn and the undrawn cold-storage chickens with one another and with a fresh chicken. The undrawn chicken had a broken right leg, which was dry and dark in color. The drawn chicken had a broken right wing, which was also dark and bloody, with an odor of putrefaction.

The undrawn chicken had a strongly fecal odor in the region of the vent, which was less marked toward the anterior end of the body. The head, however, had a strong odor. In the drawn chicken the odor was mildly rancid, somewhat butyric in character, and but slightly putrefactive.

The tissues of the undrawn chicken were soft, watery, and rather slimy, both to appearance and to touch. The same condition was

noticed for the drawn chicken, which showed in darkened areas and streaks here and there. No difference was noted between the drawn and the undrawn in the texture of the muscle.

In the undrawn fowl the spleen and kidneys appeared practically normal. The heart muscle was rather soft and the liver pale yellowish in color and easily disintegrated. In the drawn fowl the lungs were dark, bloody, and very unsightly. The heart, liver, and gizzard had been dressed and returned to the abdominal cavity. They were rather dry in appearance and somewhat darkened in color. The color of the hip bones and ends of the joints in this drawn chicken were much reddened and the subcutaneous fat, particularly near the vent, had taken on a greenish tinge. On the whole, the general physical condition of the drawn bird did not compare as favorably with the fresh chicken as did the undrawn. The bacteriological findings for these two chickens are given in the table as Nos. B. C. 4295 and B. C. 4296.

The next examination of the drawn and undrawn chickens was made after a storage period of 384 days. These chickens were thawed in an ice box protected by a sterile tin can closed by sterilized absorbent cotton. It was noticed that the normal shapes of the chickens were decidedly altered by pressure in the packing box.

The odor of the undrawn chicken was slightly rancid and somewhat fecal. The color compared favorably with that of the fresh fowl, there were, however, discolored areas over the back and the sacrum and a few blebs of gas in the subcutaneous cellular tissue. An examination of the body cavity showed soft watery viscera mixed with blood and much degenerated. The liver was pale and soft. The other organs were rather darker in color than the normal. The intestines were grayish yellow, degenerated, and very distinctly different from those of the fresh bird. Except for the condition of the lungs, the body cavity of the drawn chicken was in better condition than that of the undrawn. The heads of the long bones were dark red. As was to be expected from the macroscopic appearance of these birds, the bacteriological findings were fairly rich. They are given in detail in the table under Nos. B. C. 14 and B. C. 15.

Chickens Nos. B. C. 560 and B. C. 561 were in storage for 517 days. There had been, since the first examination, an increase in the number of infected organs, and, keeping pace with this condition, the number of species isolated increases. With such a bacteriological condition, there coexisted the following macroscopic appearance:

The skin, in both the drawn and undrawn chickens, was somewhat discolored and becoming dry, especially on the back, tips of wings, and such prominent portions. In general, the tissues were plump, though the breast of the drawn chicken was drying out. The body

cavity of the undrawn chicken showed a pale, jaundiced liver; mottled grayish-yellow lungs; darkened kidneys and degenerated intestines.

In the drawn chicken the lungs, heart, and kidneys remained in situ. They were all of a muddy, dark red appearance and there was a thin sanguineous fluid in the body cavity. In general, the interior of this bird was more degenerated than that of the undrawn, though the odor of the undrawn, especially the abdominal cavity, was distinctly fecal.

The final examination to be reported at this time was made when the chickens had been in storage 610 days. These chickens, as indicated in the table, where they are designated Nos. B. C. 695 and B. C. 696, show a more widespread, and also a more varied, bacterial invasion, than the cold-stored chickens previously examined. Their odor was rancid, the drawn having less odor than the undrawn. The tissues were generally desiccated. The skin was watery in appearance. The undrawn chicken was practically free from abrasions. The drawn had over the surface of one thigh an area of torn skin. Both chickens were discolored in the region of the vent.

Internally the undrawn chicken showed greenish-yellow fat and flabby heart; dark congested lungs, very watery; a pale yellow liver, very soft; a gizzard which was soft, flabby, and degenerated; intestines which were pale, grayish-yellow, watery, and somewhat shiny; a dark spleen and dark kidneys; a reddened bone marrow; body walls which in the region of the liver were deeply stained with bile pigment, and in the abdominal cavity itself a considerable quantity of thin, watery, bloody fluid.

In the drawn chicken the whole interior of the body cavity was dark in color and very bloody; heart and lungs dark and full of a thin, watery blood. The kidneys had practically the same appearance, all looking like pieces of old leather. This fowl, in general appearance, was not as good as the undrawn.

It is of interest to note that the species of organisms in the drawn and the undrawn fowl examined simultaneously have a very strong similarity and in many cases a coincidence. If, as is asserted by some, the bacterial invasion of the fowl in cold storage is due to the migration of the organisms from the intestinal canal into the tissues, one would expect in a drawn fowl, properly prepared, either a lack of organisms or the presence of species different from those found in the undrawn. It is quite possible, however, that the drawing of the fowl may be done in such wise that the organisms of the intestine are permitted to gain access to the viscera, in which case they would undoubtedly be found in the tissues. Gas-producing organisms, which have their ultimate habitat in the intestine, have, as will be noticed in the list of species, been isolated in certain cases. How-

ever, so widely distributed are these organisms, and so many chances would they have of coming in contact with the flesh of the chicken, that it would be difficult to assert that their presence there is due to migration from the intestine of the drawn fowl or to pollution by its contents in the process of drawing.

The pronounced increase in the number of species isolated in the course of the work here reported would indicate one of two things—either that there has been a very decided increase in the number of the organisms originally present in the flesh, enabling the methods, which at the beginning of the experiment were inadequate for their isolation, to meet the conditions present later on; or that the additional species have, in the course of the period of storage, gained access to the flesh.

It must be remembered that these chickens were prepared, placed in storage, and treated at every step of the way with a care which is not exercised under practical market conditions. With such precautions as have been taken it would seem scarcely possible that so extensive an invasion as represented by the increase in species found in the later stages of storage could have taken place in the warehouse. It seems probable, though further work is demanded on the subject, that there has been an increase in the number of the organisms in the flesh of the chickens even though the temperature at which they had been kept is far below the freezing point.

It has also been noted, in the course of this investigation, that the organisms developing after long periods of cold storage are difficult to classify. It is altogether probable that the low temperatures at which they have been kept for months is, in a measure, responsible for their variation in growth when sown upon the usual culture media. It has been quite impossible to attach a definite name to each organism since there are slight differences between individual colonies which are, however, insufficient to throw them into different groups; and they differ also from the descriptions of the organisms commonly given in the literature devoted to systematic bacteriology.

Bacteriological examination of fresh and cold-storage chickens.

FRESH CHICKENS.

No.	Date.	Description.	Time of storage.	Cultures. From—	Cultures. Results.	Species isolated.
.....	7-3-06...	Undrawn...	.....	Head wound....	+	A micrococcus, a rod form.
				Heart blood.....	0	
				Liver.....	0	
				Spleen.....	0	
				Gall bladder.....	0	
				Kidney.....	0	
				Bone marrow....	0	
				Thigh muscle....	0	
				Breast muscle...	0	
4250.....	10-19-06.	.....do.....	.....	Kidney.....	+	
				Bone marrow....	0	
				Lung.....	+	
				Thigh muscle....	0	

Bacteriological examination of fresh and cold-storage chickens—Continued.

FRESH CHICKENS—Continued.

No.	Date.	Description.	Time of storage.	Cultures. From—	Cultures. Results.	Species isolated.
4297	1-13-07	Undrawn		Heart blood	0	
				Liver	0	
				Spleen	0	
				Bone marrow	0	
				Breast muscle	0	
B. C. 13	6-11-07	do		Heart blood	0	
				Liver	0	
				Spleen	0	
				Bone marrow	0	
				Breast muscle	0	
B. C. 559	10-21-07	do	1 night	Heart blood	+	A bacillus—motile, a micrococcus.
				Liver	0	
				Spleen	+	A motile bacillus.
				Kidney	0	
				Bone marrow	0	
				Thigh muscle	0	
				Breast muscle	+	*M. pyogenes aureus*?, *B. carneus*?
B. C. 694	1-23-08	do	A few hours	Heart blood	0	
				Liver	0	
				Spleen	0	
				Kidney	+	A micrococcus.
				Bone marrow	0	
				Lung	0	
				Breast muscle	0	
				Peritoneal fluid	0	
				Intestine	+	*Streptococcus*, *M. aquatilis*?

COLD-STORAGE CHICKENS.

No.	Date.	Description.	Time of storage.	Cultures. From—	Cultures. Results.	Species isolated.
			Days.			
4252	10-19-06	Drawn	119	Heart blood	+	
				Liver	+	*M. pyog. albus.*
				Spleen	0	
				Kidney	+	*B. coli* var. ?, *B. mesentericus.*
				Bone marrow	0	
				Lung	+	*Aspergillus glaucus*, *B. coli* var. ?
				Thigh muscle	+	*B. coli* var. ?
				Peritoneal fluid	+	
4251	do	Undrawn	do	Head wound	+	*M. epidermis albus* ?, *B. fuscus* Chester.
				Heart blood	0	
				Liver	0	
				Spleen	0	
				Kidney	+	*M. candicans* ?
				Bone marrow	+	*Strep. erysipelatos*?
				Lung	+	
				Breast muscle	+	
				Peritoneal fluid	+	
				Intestines	+	Large intestine: Pink yeast, *Mucor* sp. ?, *M. aurantiacus*, *Oidium lactis*, *B. cinctus* Ravenel, *B. megatherium*, *B. putrificus* ? Small intestine: Cladosporium-like fungus.
4296	1-16-07	Drawn	215	Heart blood	0	
				Liver	0	
				Bone marrow	+	*B. fluorescens liquefaciens* ?
				Lung	0	
				Thigh muscle	0	
				Breast muscle	+	*B. fluorescens liquefaciens* ?
				Peritoneal fluid	+	*Bact. anaerogenes.*
4295	do	Undrawn	do	As in 4296	.....	Cultures and organisms as in 4296.
B. C. 15	6-11-07	Drawn	384	Thigh muscle	+	Rod, not classified.
				Breast muscle	+	Rod, not classified.

Bacteriological examination of fresh and cold-storage chickens—Continued.

COLD-STORAGE CHICKENS—Continued.

No.	Date.	Description.	Time of storage.	Cultures. From—	Cultures. Results.	Species isolated.
			Days.			
B. C. 14..	6–11–07..	Undrawn...	384.....	Heart blood.....	+	Rod, not classified.
				Liver.....	0	
				Spleen.....	0	
				Kidney.....	0	
				Lung.....	+	*Bacterium* sp. ?
				Thigh muscle....	0	
				Breast muscle...	+	*Bacterium* sp. ?, *B. fluorescens liquefaciens.*[a]
				Peritoneal fluid..	+	*Bact. avium* ? Chester.
				Intestine.....	+	*Bact. anaerogenes* ?
B. C. 561.	10–22–07.	Drawn.....	517.....	Heart blood.....	+	
				Kidney.....	+	
				Bone marrow....	0	
				Thigh muscle....	+	
				Breast muscle...	+	*B. liquefaciens, B. coli* var., *B. liquefaciens fluorescens* ?, *B. fluorescens non-liquefaciens.*
B. C. 560.	....do...	Undrawn...	.....do.....	Heart blood.....	0	
				Liver.....	+	*M. luteus.*
				Spleen.....	+	*B. raveneli* ?
				Kidney.....	+	*B. cloacae* ?, *Strep. erysipelatos* ?
				Bone marrow....	0	
				Thigh muscle....	+	*B. fluorescens non-liquefaciens, B. germinus* Ravenel ?
				Breast muscle...	+	*M. epidermis albus* ?, *B. fluorescens* ?
				Intestine.....	+	
				Crop contents...	+	*B. subtilis* ?
B. C. 696.	1–22–08..	Drawn.....	610.....	Liver.....	+	A micrococcus-like culture 687 isolated from B. C. 695.
				Kidney.....	+	
				Bone marrow....	+	
				Breast muscle...	+	
B. C. 695.	....do...	Undrawn...	.....do.....	Crop contents...	+	*Strep. enteritis, B. intestinalis* ?, *B. subtilis.*
				Heart blood.....	+	*B. alcaligenes.*
				Liver.....	+	Micrococcus culture 687.
				Spleen.....	0	
				Kidney.....	+	Micrococcus culture 687.
				Bone marrow....	+	Micrococcus culture 687, *Sarc. subflava.*
				Thigh muscle....	0	
				Breast muscle...	+	*M. cumulatus* ?, micrococcus culture 687.
				Peritoneal fluid..	+	Micrococcus culture 687, *B. coli.*
				Intestine.....	+	*Strep. enteritis, B. cloacae.*
				Pericardial fluid.	+	Micrococcus culture 687.

[a] In axillary space.

IV. MARKET COLD-STORAGE CHICKENS.

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INTRODUCTION.

The results to be set forth in the following pages represent a preliminary study, on the broadest possible lines, of the action of low temperatures on foodstuffs, more especially those rich in protein, such as meat from various animals, poultry, fish, eggs, etc. Such a study would seem to be a necessity to the well-being of the people

of the United States because of the almost universal use and enormous increase within recent years of cold-stored food products of varied kinds and from many sources.

The progressive cold-storage warehousemen are also eager for scientifically acquired data, that they may improve their product thereby. From the point of view of pure science the field for investigation would seem to be almost limitless and filled to overflowing with information most interesting and valuable to the chemist, bacteriologist, and histologist. The domain of the study is so vast, and the factors which must be considered in the correlation and interpretation of results are so numerous and far reaching in their effects, that the question has not been "What phase of the problem shall be investigated?", but, rather, "Which question can be deferred with least injury to the interpretation of the results already obtained?", since all seem to be interdependent and must be taken into account when any individual question is under consideration.

In view of the fact that this field of investigation is almost barren of reported results and because of its diversified interests, it has seemed better to make a general study of the chemical, bacteriological, and histological conditions prevailing in the case of some standard cold-stored product rather than to follow any one line of scientific work, though such a procedure would have made possible the addition of many details now waiting to be investigated. Poultry in general, and chickens in particular, have been selected as objects for study, not only because they are of enormous economic value, but, being small, highly organized, and easily obtained they would seem to be eminently suited to the present investigation.

With the extravagant advocacy of the advantages of cold storage, as claimed by some of those commercially interested on the one side, and the increasing prejudice against cold-stored foods in the minds of the laity on the other, this discussion can not deal. Suffice it to say that such contradictory statements led to the macroscopic examination of chickens which, intended for the general market, had been for varying lengths of time in cold storage, a comparison being made with fresh chickens of like breed and age.

The results of such observations would seem to demonstrate that, in appearance, odor, and texture of the flesh, long storage at low temperatures causes a variation from the normal fresh bird, and also that the changes which do occur slowly and requiring long periods are not comparable with those occurring rapidly and at ordinary temperatures. A discussion of the macroscopic changes, particularly those which might be observed by the housewife, has already been published.[a] A logical sequence of such a study is the microscopic examination of the tissues of such chickens, their chemical analysis, and a

[a] Yearbook of the U. S. Department of Agriculture, 1907.

determination of their bacterial content, on which may depend their fitness for food. The work reported will, therefore, be classified as chemical, bacteriological, and histological; the technique employed and the results obtained for these phases of the subject will be given under their respective headings; and the general discussion of the results, with, as far as is possible at this time, a correlation of the data relating to the changes, will be brought together in a final chapter.

CHEMICAL STUDIES.

RÉSUMÉ OF THE LITERATURE ON THE CHEMICAL COMPOSITION OF POULTRY.

An examination of the literature relating to the chemical composition of poultry in general, and of chickens in particular, reveals a paucity of analytical data. Such analyses as are given usually relate to the gross composition of the birds, comprising the amount of water, nitrogen, fat, nitrogen-free extractives, and ash.

König[a] has brought together in tabular form analyses made by Stutzer, Moleschott, König, Krauch and Allendorff, and Atwater and Woods, aggregating seven in all. As mean percentage values for the flesh of chickens König gives the following:

Water	72.22
Protein	21.33
Fat[b]	4.55
Nonnitrogenous extractives	0.75
Ash	1.15

In connection with a study of the meat supply in Tennessee, C. E. Wait[c] reports analyses of 20 chickens purchased in the open market in that State.

Atwater and Bryant[d] in their summary of the chemical composition of American food materials, give average data for analyses of chicken and other domestic poultry and of the gizzard, heart, etc.

Atwater[e] later reported a number of additional analyses of poultry. He examined, as a nutrition study, the carcasses of representatives of most classes of edible, domesticated birds. His analyses treat not only of the meat, but also of the giblets, the entire fowl, and the meat and giblets together, the latter being the truly edible portion. Only the analyses of the meat are available for comparison with the results obtained in this laboratory and the average data

[a] Chemie der menschlichen Nahrungs- und Genussmittel, 1903.

[b] Calculated by difference.

[c] U. S. Dept. Agr., Office of Experiment Stations, Bul. 53.

[d] U. S. Dept. Agr., Office of Experiment Stations, Bul. 28.

[e] The Composition of Poultry, Storrs Agricultural Experiment Station, 15th Annual Report, 1903.

are quoted. On one chicken separate analyses of light and dark meat were made, as will be observed in the table. There has been no attempt, however, to separate or determine the various kinds of nitrogenous bodies which go to make up the sum total of the protein. For further details those interested are referred to the original of Atwater's valuable communication.

Composition of poultry (Atwater).

Description of sample.	Water.	Protein.	Fat.	Ash.	Heat of combustion per gram.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Calories.*
2 cold-storage capons—meat	55.8	21.6	22.1	1.2	3.222
2 chickens—meat	66.9	22.6	10.1	1.1	2.209
2 chickens—broilers—meat	69.2	21.2	8.8	1.1	1.938
2 fowl—meat	58.9	20.5	19.9	1.1	2.961
1 chicken—dark meat	70.1	20.8	8.2	1.2	1.872
1 chicken—light meat	70.3	21.9	7.4	1.1	1.845

The literature on the composition of chicken fat is as unsatisfactory as that dealing with the muscle. Amthor and Zink[a] give the following values as the result of their observations on both the fat and the free fatty acids:

Chicken fat—specific gravity	0.9241
Melting point	33–40° C.
Solidifying point	21–27° C.
Iodin number	66.7
Saponification number	193.5
Reichert number	1.0
Acetyl number	45.2
Acidity (fresh)	1.2
Acidity (several months old)	2.3
Free fatty acid—specific gravity at 15° C	0.9283
Melting point	38–40° C.
Solidifying point	32–34° C.
Iodin number	64.6
Saponification number	200.8

Zaitscheck[b] gives an analysis of the fat of two chickens, one of which has been fed on corn and the other on corn and milk. His results are as follows:

Analysis of fat from corn fed, and milk and corn fed chickens (Zaitscheck).

Determination.	No. 1—corn fed.	No. 2—corn and milk fed.
Specific gravity at 30° C	0.9129	0.9153
Melting point	36° C.	38.5° C.
Solidifying point	17.4° C.	22° C.
Melting point of free fatty acids	37° C.	39.5° C.
Solidifying point of free fatty acids	34.5° C.	35.5° C.
Refraction at 40° C	25	52
Saponification number	214	216.8
Nonvolatile fatty acids	95.3	94.8
Iodin number of fats	70.6	57.6
Iodin number of fatty acids	54.4	45.4
Volatile fatty acids	0.88	0.88
Free fatty acids calculated as oleic	0.40	0.49

[a] Zts. anal. Chem., 1897, *36*: 16.

[b] Arch. ges. Physiol., 1903, *98*: 614.

Zaitscheck concludes that the food has influenced the composition of the fat, the fowl on the corn and milk ration showing values nearer those of butter fat than the results found for the chicken fed on corn alone—an observation, which, if borne out by further experimentation, is of considerable physiologic interest.

As is to be expected from the small number of analyses recorded, a lack of harmony in technique, and in the use of fowls of unknown age, breed, and previous history, there is a wide variation in results. That the analyses of the flesh of animals so highly organized as are chickens should have an exact and fixed composition is scarcely to be expected, even when breed, food, age, and the length of time between killing and examining are the same. That very wide differences do occur when such factors as have been mentioned are not taken into account is not only reasonable, but has been demonstrated by certain analyses given later in this report.

SELECTION OF MATERIAL FOR STUDY.

Since the work which has been undertaken is a comparison of the flesh of chickens kept by the aid of low temperatures for varying lengths of time, it is necessary that there should first be an acquaintance with the composition of fresh fowls of known history and of the breeds commonly found in the markets. The species most frequently kept for food purposes in the Middle States are the Plymouth Rock (white and barred), White Wyandotte, Rhode Island Red, and Leghorn. The last-named species is especially valuable for egg production. The birds are too small and the breast muscles are not sufficiently well developed to render them as desirable for market purposes as are the other breeds, but because of their laying qualities they are fairly plentiful, and young cocks and old hens are therefore killed off and sold for food.

As the cold-stored fowls which are to be discussed were taken from regular market stock and are, almost exclusively, of the Plymouth Rock variety, or its crossbreeds, the discussion of the composition of fresh chickens will be limited accordingly.

The birds kept for standards of comparison were fed chiefly on Indian corn, milk, and clean table scraps. They were regularly and abundantly fed, were supplied with pure water, and their runs were placed in an orchard having a southern exposure, this environment insuring rapid growth and tender flesh. For 24 hours previous to killing all food was withheld, but water in plenty was provided. They were killed by puncturing the spinal cord by way of the mouth, and were bled well, dry picked, and the animal heat was quickly extracted. They were kept for from 18 to 24 hours at the temperature of an ordinary refrigerator before the analysis was begun, that being the period required for a frozen chicken to thaw in a house refrigerator. All were undrawn.

The history of the cold-storage chickens is unknown up to the beginning of storage, the date of which is known. They were first placed in a cold freezer having a temperature of from 4° to 6° F. until solidly frozen, which requires about 48 hours; then they were transferred to a temperature of approximately 13° F. All of the market cold-storage chickens reported at this time have been treated similarly. They were removed from the warehouse about 24 hours before the analysis was begun and were allowed to thaw at the temperature of an ice box. Occasionally, especially in the case of large birds, there would be enough ice in the tissues when the examination was begun to cause the knife to grit, but usually they were simply very cold to the touch.

One chicken only—No. 69, in storage two years—was thawed in the usual commercial fashion, namely, by soaking in cold water. Since such treatment prevents any accurate idea of the bacterial content of the flesh itself, and as a bacterial, a histological, and a chemical examination of each fowl was desired, dry thawing was adopted. Such a procedure gave a greater proportion of solid matter in the flesh than was obtained in either the fresh or the soaked chickens, because of the marked drying of the tissues while in storage.

CHEMICAL TECHNIQUE.

The methods of chemical analysis employed were, as nearly as possible, those officially recommended and commonly used in flesh analysis. A brief outline of the procedure followed is given. The chemical analyses were made by J. S. Hepburn and John I. Burrell.

Preparation of Sample.

When the birds were large enough, only one was used for analysis. When small, two were used, care being taken to thoroughly mix the tissue.

The flesh of the chicken was prepared for analysis by separating it from the bones and the skin, removing as completely as possible the fat between the muscles and also the large tendons, which in the leg and wing muscles of the chicken are easily stripped loose from the meat. Their absence is a factor too small to influence analytical results, but their presence renders the grinding and accurate sampling of the meat more difficult. The muscles of the thighs and legs were classed as dark meat, of the breast and upper wings as light meat. The dark and light portions were put separately through a meat grinder until the particles were very finely divided, which necessitated ordinarily three grindings. It was found undesirable to grind the flesh too finely, since the shaking required for the extraction of the soluble material caused an emulsion which it was practically impossible to filter.

The skin, with the adhering subcutaneous fat, was mixed with the fat separated from the viscera and the abdominal cavity and that stripped off of the muscles, ground in the meat cutter until fine, and used for the study of the fat of the fowl.

Muscle Determinations.

The general analysis of the muscle was based on the determination of water, fat, ash, total nitrogen, and total solids. The nitrogenous portion was further investigated for the nitrogen soluble in water, and in this aqueous extract was determined the coagulable nitrogen, albumose nitrogen, and amido acid nitrogen, the peptone nitrogen and the nitrogen insoluble in water being found by difference.

For the determination of the total solids, water, fat, and ash in the muscle, a homogeneous well-mixed sample of the meat was weighed in a tightly stoppered weighing bottle, and portions, of approximately 10 grams each, were transferred to appropriate containers, as desired.

TOTAL SOLIDS AND WATER.

For the total solids and water a 10-gram portion was dried at 100° C. in a tared lead bottle cap from 2 to 3 inches in diameter. It being practically impossible to dry a sample of such material as chicken meat to constant weight, because of the oxidation of the substance after the removal of the last traces of water, the sample was reweighed at short intervals and a gain in weight taken as an indication of the absence of moisture. This gain was ordinarily not more than a few tenths of a milligram. Occasionally, however, it was several milligrams.

FAT.

The quantity of fat in the muscle material itself was found by extracting the dry residue from the water and total solids determination with Squibb's ether. This was readily accomplished by cutting up the lead cap, with its contents, into small pieces and placing the mixture in the thimble of a Knorr extractor, which was then, with the requisite amount of ether, heated for 16 hours on an electric heater. When the extraction was completed, the ether was evaporated and the fat dried at 100° C. until the moisture was removed, which was indicated by a slight gain in weight, as in the case of the total solids. The weighings here were at intervals of from 2 to 3 hours.

ASH.

For the determination of the amount of ash two or three grams of the sample were dried in a platinum crucible, then heated in a muffle until completely charred, allowed to cool, and extracted sev-

eral times with hot water. The carbon insoluble in water, and the small ashless filter paper required for the extraction, were returned to the crucible and the ignition repeated, using ammonium nitrate, if necessary, to oxidize the last particles of carbon. To the grayish mass the aqueous extract was then added, dried on a water bath, and ignited in a muffle until the ash was clean.

NITROGENOUS CONSTITUENTS.

The study of the nitrogenous constituents was carried out as follows: The total nitrogen of the two kinds of muscle was determined by the Gunning modification of the Kjeldahl method. For the study of the water soluble nitrogen a portion of the finely divided red or white meat, weighing 60 grams, was put into a tall slender bottle of 500 cc capacity, constructed to fit a centrifuge capable of carrying 1 liter of material; 300 cc of water were added and the flask gently shaken for 15 minutes. The movement was merely sufficient to keep the particles of meat in motion and the composition of the extract homogeneous. Forcible shaking caused an emulsion to form, as did the very fine grinding of the tissue. After shaking for the required length of time the flask was rotated in an electric centrifuge for 20 minutes, which caused the heavier particles to settle in a compact mass and permitted the decantation of the supernatant liquid, which was then filtered through paper. The extraction, as outlined, was repeated with portions of 300 cc of water until the filtrate was practically proteid free, as indicated by the biuret reaction. The attainment of this result required ordinarily a volume of 1,500 cc to 2,500 cc. To guard against bacterial decomposition thymol was added both to the flesh and to the extract, and to inhibit, so far as possible, the action of the naturally occurring enzymes of the meat, the solution and the meat itself were kept cold, ice being used when necessary.

The extraction of the white meat was a much simpler operation than the extraction of the dark meat. The latter did not settle as compactly in centrifuging, filtered more slowly, and persisted in showing a distinct biuret reaction for a considerable time after the white meat was free of water-soluble proteid. In fact, certain fowls, more especially those which have been in cold storage for long periods of time, never showed a red meat entirely free from water-soluble nitrogen. In such cases the question of the error due to long manipulation and enzyme action, involving a rise in the actual quantity, had to be considered. It was found by experiment that after long extraction of such tissue a point was reached when a very faint biuret reaction, which did not apparently diminish, persisted indefinitely. Such extractions were halted after about 26 hours, it being believed that a greater error would result in the gain of what had been originally

insoluble material than in the loss of the preformed water-soluble nitrogen. The total extract of the muscle was made up to a definite volume and neutralized to litmus paper with tenth-normal sodium hydrate.

Duplicate portions of 100 cc each were transferred to beakers, evaporated to very small bulk (about 10 cc), and transferred with the aid of sulphuric acid to Kjeldahl flasks for the determination of total nitrogen soluble in water. Duplicate portions of 350 cc were heated on the water bath in order to determine the amount of coagulable nitrogen present. It was found better for the complete separation of the proteid to evaporate before filtering to about 100 cc. The coagulum of the white meat separated more easily and in a much more compact mass than did the coagulum of the dark meat. Both were difficult to wash free of proteid material.

The coagulum and the filter paper were transferred to a Kjeldahl flask for the determination of the amount of nitrogen. The filtrate from the coagulum, having been considerably increased in volume by the washings, was reduced to 100 cc. Fifty cubic centimeters of this were taken for the determination of albumose, which was made according to the method of Bömer.[a] Concentrate the liquid to 30 cc and allow it to cool; add 1 cc of 50 per cent sulphuric acid, saturate with zinc sulphate, warm on a water bath with stirring until clear, and allow to stand 12 hours for the separation and deposition of the precipitate. Filter cold and wash with a saturated solution of zinc sulphate acidified with sulphuric acid. The determination of the nitrogen is made as usual.

In the other 50 cc portion of the filtrate from the coagulated proteid the amido acids were determined according to the method of Bigelow and Cook,[b] which, briefly stated, consists in the addition of 15 grams of sodium chlorid to 50 cc of the amido acid containing extract, chilling thoroughly in an ice box, then adding 30 cc of a 24 per cent solution of tannic acid, making the volume up to 100 cc, and allowing the whole to stand at the temperature of an ice box for 24 hours, after which the precipitate is filtered off. The solution is kept cold meanwhile, and the nitrogen in 50 cc of the filtrate is determined according to the usual Kjeldhal procedure, except that the addition of potassium sulphate is unnecessary, because of the large amount of sodium chlorid present, and care must be taken during the early part of the oxidation that the escaping hydrochloric acid does not cause the material to foam out of the flask.

Since tannic acid invariably contains a certain amount of nitrogen, a blank must be run with every experiment.

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[a] Zts. anal. Chem., 1895, *34*: 562.

[b] J. Amer. Chem. Soc., 1906, *28*: 1485.

Fat Determinations.

The study of the fat consisted in the determination of the iodin number, the saponification number, the acid value, ester value, the Hehner number, and the index of refraction.

The fat of the fowl, prepared as previously described (page 63), was heated for 2 days in the thimble of a Soxhlet extractor, with a petroleum ether having a boiling point of between 40° and 60° C. If any solid particles separated after the extraction the solution of the fat was filtered through paper, and the ether was then distilled off. Drying the fat, with the small amount of adhering tissue, before extracting, was not practiced. The quantity of tissue was very small, because the mechanical separation was carefully made, and it was deemed better to risk the mixture of a certain amount of nitrogenous extractives with the fat than to subject it to the splitting produced by the action of air and heat. It is proposed to make a study of these two procedures to determine their relative accuracy. It was found exceedingly difficult to remove the last traces of petroleum ether without altering, to a greater or less extent, the composition of the fat itself. The result was accomplished best by allowing the fat, freed as far as possible from petroleum ether by means of distillation, to stand in a vacuum desiccator over calcium chlorid freshly ignited and acid free. It was found that sulphuric acid, in a vacuum desiccator, very materially altered the value of the fat, probably owing to the absorption of a certain amount of sulphuric acid vapor.

Though it is generally agreed among bacteriologists that pure dry fat is not a culture medium for bacteria, the precaution was nevertheless taken to preserve the samples of fat, even for the short interval between the distillation of the petroleum ether and their further analysis, in sterile flasks, plugged with sterile cotton, and the transfer of the material when necessary was made with sterile pipettes. Because of the action of light and air on pure fat the analysis of the samples was conducted with all possible haste.

The iodin number was determined according to the official Hanus method.[a] For the saponification number the official method was also followed, and the Hehner number was determined as officially recommended.[a] The acids, however, were dried at 100° C. until a gain in weight occurred, when the minimum weight was taken as expressing the quantity of acid.

For the acid value 4 or 5 grams of the fat were weighed in a 250 cc Erlenmeyer flask; 100 cc of a 15 per cent sodium chlorid solution were added, and the flask was immersed in hot water until the fat was liquefied. The free acid was then titrated with tenth-normal sodium hydroxid, using phenolphthalein as an indicator. Vigorous

[a] U. S. Dept. Agr., Bureau of Chemistry, Bulletin 107, pp. 136–139.

and frequent shaking is necessary to determine the permanence of the delicate but distinct pink which indicates the end of the titration. For convenience of expression the acid value has been calculated also as oleic acid, which is readily accomplished, since the acid value is expressed as milligrams of potassium hydrate. The difference between the acid value and the saponification number represents the ester value.

The refractive index[a] was determined independently by three or four observers, and the mean of all of the observations taken. An Abbe refractometer was used. The readings were taken at a temperature between 30° and 40° C. and corrected to 35° C. by the use of the factor 0.000365 for 1° C.

ANALYSES OF FRESH CHICKENS.

The values obtained in the chemical study of fresh chickens are given in Tables A to D. In Table B the comparatively low acid value shown by this particular fat is striking, and in Table C the relatively large amount of protein in the light meat as compared with that in the dark and its greater solubility in water are to be noted.

Throughout all the analyses there are decided differences between the individuals themselves. Whether such variations can be traced to definite conditions it will take many more analyses and much study to determine. It is believed, however, that the data on these fresh chickens are sufficient to show that certain differences do exist between them and chickens preserved for varying lengths of time in cold storage.

(*A*) *Percentage composition of fresh chicken muscle.*

Sample.	Kind of meat.	Water.	Fat.	Ash.	Protein (N × 6.25).	Kreatin (N × 3.11).	Total solids.	Sum of constituents determined.
No. 66. Plymouth Rock broiler	Light	75.50	0.49	1.17	23.46	1.10	24.50	97.92
	Dark	71.75	2.40	1.21	21.40	0.827	28.25	101.33
No. 68. Plymouth Rock young roaster or broiler	Light	75.73	0.17	1.33	21.84	1.01	24.27	100.08
	Dark	75.86	1.38	1.49	21.07	0.64	24.14	100.44
No. 73. Plymouth Rock roaster	Light	73.30	0.51	1.24	22.52	0.920	24.70	100.92
	Dark	74.48	2.88	1.18	20.69	0.743	25.52	99.97
No. 78. Rhode Island Red roaster	Light	73.56	0.98	1.26	23.50	1.01	26.44	100.31
	Dark	73.02	2.99	1.35	23.13	0.64	26.99	101.13
No. 86. Rhode Island Red large broiler	Light	75.01	0.53	1.21	21.95	1.02	24.99	99.13
	Dark	75.94	2.15	1.13	19.77	0.796	24.06	99.78

[a] U. S. Dept. Agr., Bureau of Chemistry, Bulletin 107, p. 131.

(B) Analysis of fat of fresh chicken.

Determinations.	Plymouth Rock broiler, No. 66.	Plymouth Rock broiler, No. 68.	Plymouth Rock broiler, No. 73.	Rhode Island Red roaster, No. 78.	Average.
Iodin number	62.6	71.2	62.7	61.1	64.4
Saponification number	182.2	176.7	190.2	176.8	181.4
Acid value	0.5	1.5	0.8	0.5	0.7
Ester value	181.7	175.2	189.4	176.3	180.6
Per cent of free acid calculated as oleic	0.25	0.76	0.40	0.25	0.41
Hehner number	86.8	84.25	88.7	85.79	86.36
Index of refraction at 35° C				1.4566	

(C) Percentage of nitrogen in muscle of fresh chicken.

LIGHT MEAT.

Determinations.	No. 66.	No. 68.	No. 73.	No. 78.	No. 86.	Average.
Total nitrogen	4.11	3.82	3.90	4.07	3.84	3.94
Total nitrogen in aqueous extract	0.920 [a] **22.40**	0.954 **25.00**	0.995 **25.5**	0.922 **22.65**	0.923 **24.03**	**23.91**
Coagulable nitrogen in aqueous extract	0.338 **8.23**	0.408 **10.7**	0.534 **13.7**	0.402 **9.90**	0.371 **9.66**	**10.42**
Albumose nitrogen	0.0269 **0.655**	0.0290 **0.775**	0.0181 **0.465**	0.0188 **0.462**	0.0392 **1.02**	**0.675**
Amido acid nitrogen	0.355 **8.65**	.....	0.296 **7.90**	0.325 **8.00**	0.328 **8.55**	**8.27**
Peptone nitrogen (by difference)	0.200 **4.87**	.....	0.147 **3.76**	0.177 **4.35**	0.185 **4.81**	**4.19**
Nitrogen insoluble in water (by difference)	3.19 **77.60**	2.86 **74.8**	2.90 **74.5**	3.15 **77.5**	2.917 **76.00**	**76.09**

DARK MEAT.

Determinations.	No. 66.	No. 68.	No. 73.	No. 78.	No. 86.	Average.
Total nitrogen	3.69	3.58	3.55	3.91	3.42	3.63
Total nitrogen in aqueous extract	0.659 [a] **17.85**	0.704 **19.68**	0.639 **18.00**	0.569 **14.30**	0.645 **18.85**	**17.73**
Coagulable nitrogen in aqueous extract	0.291 **7.89**	0.320 **8.93**	0.331 **9.34**	0.252 **6.45**	0.262 **7.66**	**8.05**
Albumose nitrogen	0.0235 **0.637**	0.0205 **0.573**	0.0112 **0.316**	0.0103 **0.263**	0.0215 **0.628**	**0.483**
Amido acid nitrogen	0.266 **7.21**	.....	0.239 **6.75**	0.208 **5.33**	0.256 **7.48**	**6.69**
Peptone nitrogen (by difference)	0.087 **2.36**	.....	0.057 **1.60**	0.099 **2.56**	0.106 **3.1**	**2.39**
Nitrogen insoluble in water (by difference)	3.03 **82.00**	2.88 **80.5**	2.91 **82.1**	3.34 **85.5**	2.77 **81.3**	**82.27**

[a] All figures in bold-faced type are percentages based on total nitrogen.

(*D*) *Nitrogenous constituents of fresh chickens (calculated on water- ash- fat-free basis).*

LIGHT MEAT.

Determinations.	No. 66.	No. 68.	No. 73.	No. 78.	No. 86.	Average.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Total nitrogen	19.74	16.77	16.95	16.80	16.38	17.32
Total nitrogen in aqueous extract	4.43	4.19	4.33	3.81	3.94	4.14
Coagulable nitrogen in aqueous extract	1.626	1.79	2.32	1.66	1.58	1.77
Albumose nitrogen	0.110	0.130	0.0788	0.0778	0.167	0.112
Amido acid nitrogen	1.46	.....	1.29	1.34	1.40	1.37
Peptone nitrogen (by difference)	0.962	.....	0.639	0.409	0.790	0.7
Nitrogen insoluble in water (by difference)	15.31	13.97	12.62	12.99	12.42	13.46

DARK MEAT.

Total nitrogen	13.90	16.65	16.50	17.25	16.42	16.14
Total nitrogen in aqueous extract	2.49	3.27	2.97	2.51	3.10	2.86
Coagulable nitrogen in aqueous extract	1.10	1.49	1.62	1.11	1.26	1.31
Albumose nitrogen	0.0890	0.0953	0.0472	0.0455	0.101	0.075
Amido acid nitrogen	1.008	.....	0.968	0.916	1.23	1.030
Peptone nitrogen (by difference)	0.328	.....	0.265	0.437	0.509	0.384
Nitrogen insoluble in water (by difference)	11.42	13.38	13.53	14.74	13.32	13.27

ANALYSES OF COLD-STORED CHICKENS.

The composition of the muscle and of the fat of cold-stored fowls is given in Tables A to D, beginning on page 73. Each analysis, with the exception of No. 69, from a bird in storage 2 years, is based upon the mixed flesh and fat of two chickens.

Chickens No. 84 and No. 85 had been cold-stored for 14 months. They were obtained, as were all the fowls here reported, from the regular stock of a reputable dealer. They were rather carefully packed in a shallow box, but not individually wrapped. When thawed they presented a dried appearance, the skin was dense, the feather papillæ flat, and the color faintly greenish—a color differing markedly from that of a fresh chicken—yet, in comparison with chickens stored for longer periods, they were good-looking birds. The muscles of the breast were somewhat deeper in color than when fresh and the texture was noticeably different, having a slippery feel. The muscle fibers presented an uneven surface when cut, rather than the sharp edge given by a fresh muscle, and, though not markedly dry when first exposed, they became so very quickly—indeed after an hour or two a result totally distinct from that on the exposed fresh muscle was obtained. All of these changes were intensified in the

muscles of the inner thigh, especially the color changes. The fat had become opaque and deeper in color, most noticeably between the leg muscles.

No odor of putrefaction was observed, but there was a distinct smell, which seems to be characteristic of cold-stored as compared with fresh fowls, and which suggests acrolein. This, when chickens have been long in storage, is sufficiently strong to be decidedly irritating to both eyes and nasal membranes.

The viscera were in fair condition, as far as could be detected by macroscopic observation, except that the walls of the intestines were much thinner than is normal, and the kidneys had greatly softened.

These chickens contain an average of 72.03 per cent of water, as compared with 74.41 per cent in the fresh birds, a loss of approximately 2.5 per cent. It is of interest to observe that, though the fresh chickens have an almost equal content of water in the light and dark meat, the fowls in storage for 14 months show a more pronounced drying of the dark muscle. Since the sale of chickens is, for food purposes, exclusively by weight, and since these cold-stored birds take up by soaking a large proportion of the water lost by evaporation, one can readily understand the preference of the retailer to soak the fowls before offering them to the public.

The fat content, as specified in Table A, is rather in the nature of a factor upon which to calculate other results than an actual representation of its quantity in the muscle, because the adhering fat from the tissue was picked off as cleanly as possible, and only that in the individual muscles was left. Such hand cleaning, however, is not absolute, though there is a fair agreement between results.

As one would expect from the loss of moisture, the relative amount of nitrogen has slightly increased, the average for both light and dark meat in the fresh fowl standing at 21.93 per cent when calculated as protein and for the stored fowls 23.67. The distribution of this nitrogen among the various classes of protein and amido bodies is given in Table C, page 73. The figures in ordinary type represent the percentage amounts of nitrogen; those in bold-faced type indicate the relative proportion which such quantities bear to the total nitrogen, and the figures in italics are the mean values of the analyses of duplicate samples.

The analysis of the fat of the normal chickens would indicate for it a fairly constant composition. So widely do the values for the storage chickens vary that it is not possible to take the mean of even duplicate samples. But different as the results are, the trend of the changes in all of the stored market chickens seems to be along definite lines, namely, a lowering of the iodin number, the saponification number, the ester value, and the Hehner number, and a very marked increase in the quantity of free acid.

CHICKEN AFTER STORAGE AT 13° F. FROM DECEMBER 24, 1903, TO FEBRUARY 6, 1908.

Samples No. 69 and No. 79 represent chickens from a box kept in storage for 1 year, then thawed and prepared for sale, but because they did not sell and fearing that they would spoil before they could be disposed of the dealer put them back again into storage, where they remained another year. They were then examined—No. 69 after thawing in water and No. 79 in cold air.

The external appearance of these fowls was very different from that of fresh ones. The skin was leathery, inelastic, tightly drawn, and discolored, especially over certain portions, and even soaking in water did not loosen it from the prominent bones where it had been stretched. The muscles of the breast were markedly dried, in some places so much so that they were light-brown fibrous masses, quite unrecognizable in themselves as chicken muscle. Such degeneration had not, however, penetrated to the deep muscles. The color of the muscles of the inner thigh and of the fat was also very different from the colors commonly characterizing these tissues in fresh birds. Deep reds, verging on purplish or brownish, predominated in the muscles, and the fat was bright orange. The biting odor, noticed in the 14-months' chickens, was intensified in these 2-year specimens, and after a few hours in the air an indication of putridity could be detected.

As previously stated No. 69 was thawed by soaking in water; hence, in the percentage values of water, total nitrogen, etc., it is not comparable with the others of the series. The relative proportions of the various nitrogenous constituents are not, however, interfered with and the mean of the two analyses can be taken.

Except for an increase in peptone in the light meat, a decrease in amido acids, and a decrease in coagulable and albumose nitrogen in the dark meat, the analyses show a close similarity between these chickens and the fresh ones. It is rather remarkable, however, to find that the total nitrogen in the aqueous extract and also the nitrogen coagulable by heat is less than in the fresh chicken. Still, because of the commercial thawing and handling, with its train of unknown conditions and results, one must come most cautiously to conclusions of any kind.

The analysis of the fat of No. 69 is incomplete, because the fowl was so thin that a sufficient quantity for the determination of all the values could not be obtained. For No. 79, where two chickens were taken, material was more plentiful. Between the fat analysis of Nos. 69 and 79, as between the two samples in storage 14 months, are wide variations. The same general tendencies, however, are also seen in these analyses.

Samples Nos. 82 and 83 represent chickens which have been cold-stored for a period of 4 years. Plates IV and V are photographs of these chickens taken after thawing in cold air. Plate IV shows well the rigidity of the fowl, since it was hung on a string slipped under

the legs without any other support and has still retained the position occupied when packed in the case. Plate V shows another chicken which had been massaged and manipulated until it was as flexible as such treatment would make it. The lack of success, however, is well illustrated by the position of the neck. These photographs show well the drying of the tissues, as indicated by concavities on legs and back, where the fresh chicken is normally well rounded. The texture of the skin is leathery in the extreme and yet brittle. All the changes noticed in the 2-year chickens are greatly exaggerated in those kept for 4 years, especially the color, which is of a greenish hue throughout, and when removed the under surface of the skin showed a color similar to the outside. The muscles of the breast were dried to a parchment-like covering, easily mistaken for the skin, and in some places the drying reached to the bone. Where this was not the case the color of the muscle had changed to a rust red. While the inner thigh muscles did not show such marked evidences of desiccation, they were more discolored. Deep browns and purplish reds predominated, while the shrunken bands of fat between the muscles were a deep brown-orange. The body wall overlying the intestines was distinctly green; the viscera were in bad condition, the liver mushy, and the kidneys a thin paste. The intestinal walls were mere remnants of membranes, from which nearly all the cellular elements had disappeared.

A proteolytic change in the muscles of market cold-stored fowls is evidenced by the changes occurring in the relative proportions of the various proteid constituents. This is sometimes shown as an increase in the amount of nitrogen soluble in water, which may be accompanied by a rise in the nitrogen coagulated by heat; or there may be a change in the relative amounts of albumose, peptone, and amido acids. These changes, indicated by the chemical data, are substantiated by the histological and bacteriological findings. It must be remembered in this connection that the market cold-stored chickens are to be considered as individuals and compared with the fresh fowls rather than with one another. For example, the chickens in storage for two years, having been thawed by soaking in water and refrozen under market conditions, are not comparable with those frozen continuously and thawed once in cold air. Similarly it is probable that the bacteriological condition of Nos. 84 and 85, examined after fourteen months' storage, was worse when put into storage than was that of Nos. 82 and 83, and hence conclusions in regard to progressive changes can not be drawn from such data.

The analysis of the fat of the two birds numbered 83 gives the highest acid value yet reached, corresponding to 27.04 per cent of free oleic acid. The iodin number is the lowest, but the Hehner number, though below the normal, is not exaggeratedly so.

CHICKEN, MASSAGED AND MADE AS FLEXIBLE AS POSSIBLE, WITHOUT SOAKING, AFTER STORAGE FROM DECEMBER 24, 1903, UNTIL FEBRUARY 6, 1908.

(*A*) *Percentage composition of cold-storage chicken muscle.*

Number.	Time of storage.	Kind of meat.	Water.	Fat.	Ash.	Protein (N × 6.25).	Kreatin (N × 3.11).	Total solids.	Sum of constituents determined.
	Months.								
84	14	Light	73.67	0.27	1.10	23.01	1.16	26.33	99.21
		Dark	72.16	1.93	1.27	23.16	0.852	27.85	99.37
85		Light	72.22	0.27	1.14	24.36	1.10	27.78	99.16
		Dark	70.09	2.85	1.25	24.55	0.877	29.91	99.61
69[a]	24	Light	74.43	0.40	0.95	23.00	0.836	25.57	99.61
		Dark	73.11	2.04	1.07	22.78	0.761	26.89	99.76
79		Light	72.60	0.83	1.06	25.10	0.569	27.40	100.15
		Dark	69.52	2.93	1.21	25.50	0.715	30.48	99.87
82	48	Light	71.42	0.48	1.39	24.78	0.945	28.58	99.01
		Dark	67.10	3.80	1.35	26.28	0.914	32.90	99.44
83		Light	65.87	0.19	1.48	26.91	1.41	34.13	95.86
		Dark	65.48	3.10	1.59	27.00	0.801	34.52	97.97

[a] Thawed by soaking.

(*B*) *Analysis of fat of cold-storage chickens.*

Determinations.	Storage 14 months.		Storage 2 years.		Storage 4 years.	
	No. 84.	No. 85.	No. 69.[a]	No. 79.	No. 82.	No. 83.
Iodin number	58.8	48.1	68.7	61.0	63.6	47.1
Saponification number	131.7	162.2	.....	161.1	172.8	167.6
Acid value	48.6	17.5	13.7	4.0	4.6	53.8
Ester value	83.1	144.7	.....	157.1	168.2	113.8
Per cent of free acid calculated as oleic	24.43	8.80	6.90	2.01	2.31	27.04
Hehner number	61.78	73.69	.....	77.09	81.65	75.81
Index of refraction	1.4566	1.4576	.....	1.4538	1.4584	1.4568

[a] Thawed by soaking.

(*C*) *Percentage of nitrogen in muscle of chicken.*

LIGHT MEAT.

Determinations.	Storage 14 months.		Storage 2 years.		Storage 4 years.	
	No. 84.	No. 85.	No. 69.[a]	No. 79.	No. 82.	No. 83.
Total nitrogen	4.06	4.27	3.95	4.20	4.27	4.76
	4.16				*4.51*	
Total nitrogen in aqueous extract	1.176	1.175	0.886	0.963	1.194	1.470
	28.96	**27.51**	**22.45**	**22.90**	**27.90**	**34.50**
	28.23		*22.67*		*31.20*	
Coagulable nitrogen in aqueous extract	0.581	0.571	0.341	0.490	0.635	0.764
	14.31	**13.43**	**8.65**	**10.78**	**14.87**	**16.09**
	13.87		*9.71*		*15.48*	
Albumose nitrogen	0.0588	0.0514	0.0198	0.0354	0.0258	0.0643
	1.44	**1.20**	**0.502**	**0.844**	**0.604**	**1.353**
	1.32		*0.673*		*0.978*	
Amido acid nitrogen	0.376	0.356	0.269	0.246	0.304	0.454
	9.26	**8.35**	**6.82**	**4.36**	**7.01**	**9.55**
	8.80		*5.59*		*8.29*	
Peptone nitrogen (by difference)	0.161	0.194	0.256	0.061	0.229	0.188
	3.96	**4.55**	**6.48**	**3.83**	**5.36**	**3.85**
	4.25		*5.15*		*4.60*	
Nitrogen insoluble in water (by difference)	3.04	3.28	3.08	3.24	3.08	3.29
	74.87	**76.9**	**78.0**	**77.1**	**72.20**	**69.10**
	75.88		*77.55*		*70.65*	

[a] Thawed by soaking, average omitted for total nitrogen.

(*C*) *Percentage of nitrogen in muscle of chicken*—Continued.

DARK MEAT.

Determinations.	Storage 14 months.		Storage 2 years.		Storage 4 years.	
	No. 84.	No. 85.	No. 69.[a]	No. 79.	No. 82.	No. 83.
Total nitrogen	3.98	4.21	3.89	4.31	4.50	4.61
	4.09				*4.55*	
Total nitrogen in aqueous extract	0.824	1.014	0.596	0.639	0.869	0.866
	20.75	**24.08**	**15.30**	**14.80**	**19.30**	**18.80**
	22.41		*15.05*		*19.05*	
Coagulable nitrogen in aqueous extract	0.485	0.547	0.272	0.302	0.445	0.434
	12.18	**12.99**	**6.99**	**7.00**	**9.88**	**9.40**
	12.53		*6.99*		*9.64*	
Albumose nitrogen	0.0413	0.055	0.0222	0.0461	0.0433	0.0443
	1.03	**1.30**	**0.570**	**1.70**	**0.962**	**0.960**
	1.16		*1.135*		*0.961*	
Amido acid nitrogen	0.274	0.282	0.245	0.230	0.294	0.290
	6.89	**6.70**	**6.30**	**5.34**	**6.53**	**6.29**
	6.79		*5.82*		*6.41*	
Peptone nitrogen (by difference)	0.0237	0.356	0.057	0.061	0.087	0.098
	0.59	**3.08**	**1.47**	**1.41**	**1.93**	**2.12**
	1.83		*1.44*		*2.02*	
Nitrogen insoluble in water (by difference).	2.97	3.32	3.29	3.67	3.63	3.74
	74.6	**78.9**	**84.5**	**85.30**	**80.60**	**81.00**
	76.7		*84.9*		*80.80*	

(*D*) *Percentage of nitrogenous constituents.*

[Calculated on water- ash- and fat-free basis.]

LIGHT MEAT.

Determinations.	Storage 14 months.		Storage 2 years.		Storage 4 years.	
	No. 84.	No. 85.	No. 69 [a]	No. 79.	No. 82.	No. 83.
Total nitrogen	16.30	16.20	16.30	16.45	16.00	14.65
	16.25				*15.32*	
Total nitrogen in aqueous extract	4.72	4.47	3.66	3.77	4.475	4.52
	4.59		*3.71*		*4.49*	
Coagulable nitrogen in aqueous extract	2.33	2.18	1.41	1.94	2.38	2.35
	2.25		*1.67*		*2.31*	
Albumose nitrogen	0.236	0.195	0.082	0.138	0.0957	0.197
	0.215		*0.110*		*0.146*	
Amido acid nitrogen	1.51	1.35	1.01	0.727	1.138	1.395
	1.43		*0.868*		*1.26*	
Peptone nitrogen (by difference)	0.645	0.737	1.06	0.975	0.858	0.578
	0.691		*1.01*		*0.718*	
Nitrogen insoluble in water (by difference).	12.20	12.48	12.64	12.30	11.52	10.12
	12.34		*12.47*		*10.82*	

DARK MEAT.

Total nitrogen	16.20	16.32	16.38	16.40	16.24	15.42
	16.26				*15.83*	
Total nitrogen in aqueous extract	3.34	3.94	2.51	2.43	3.13	2.90
	3.64		*2.47*		*3.01*	
Coagulable nitrogen in aqueous extract	1.97	2.12	1.10	1.15	1.60	1.45
	2.04		*1.12*		*1.52*	
Albumose nitrogen	0.167	0.213	0.0937	0.1752	0.156	0.148
	0.190		*0.1344*		*0.152*	
Amido acid nitrogen	1.111	1.093	1.03	0.876	1.06	0.970
	1.102		*0.953*		*1.015*	
Peptone nitrogen (by difference)	0.202	0.504	0.240	0.232	0.314	0.338
	0.353		*0.236*		*0.326*	
Nitrogen insoluble in water (by difference).	12.10	12.88	13.85	13.98	13.10	12.52
	12.49		*13.91*		*12.81*	

[a] Thawed by soaking, average omitted for total nitrogen.

BACTERIOLOGICAL STUDIES.

TECHNIQUE.[a]

The aim of the work having been to study not only qualitatively, but also quantitatively, the organisms found in the flesh of chickens, it was necessary to develop methods which would be available for the study of both phases of the subject. Since the number of organisms in the flesh, as well as their variety, is of great importance from the sanitary point of view, much stress has been laid upon the methods used for the enumeration of the bacteria.

This phase of the work necessitated, of course, the use of solid media. As the variety of possible organisms was great it was not advisable to attempt to use a number of special media, but rather to select a few which would be generally available for the great majority of bacteria. Therefore, plain agar neutral to phenolphthalein, litmus-lactose-agar, and nutritive glucose gelatin which was 1 per cent acid were used in routine work.

Because of the great variation in the number of organisms developing at different temperatures[b] the plates were incubated at 37°, 20°, and −1.67° C. It was found in the progress of this work that the removal of a small amount of tissue with a sterile platinum wire or loop, such as usually suffices for the study of infected flesh, generally gave negative results; and the removal of several such pieces gave results which were not comparable, though every precaution was taken to prevent contamination and to treat each piece exactly as the others were treated. It became necessary, therefore, to remove larger pieces of tissue and to develop some scheme by which these could be disintegrated in a fluid medium which would not affect the organisms and which should have a quantitative value in order that the number of bacteria might be determined for a definite weight of material. Numerous experiments, with varying quantities of tissue and forms of sterile containers, finally evolved the following technique, which has yielded results that are fairly constant and are comparable.

Several small pieces of flesh, aggregating from 0.3 to 0.7 of a gram, are removed with sterile instruments from an uncontaminated portion of the tissue which is being investigated, and are placed in an Erlenmeyer flask of 25 cc capacity, containing a sufficient number of thin pieces of broken glass to cover the bottom, and which, after sterilization, had been accurately weighed. Reweighing gave, by difference, the weight of the tissue. A measured quantity of a suit-

[a] The bacteriological examinations reported have been made by E. Q. St. John.

[b] Pennington, Bacterial Growth and Chemical Changes in Milk at Low Temperatures, J. Biol. Chem., 1908, *4*: 353.

able liquid carrier for the organisms, generally physiological salt, was then put into the flask and the whole was shaken with sufficient vigor to agitate both the pieces of glass and the tissue. Since the portions of the latter were not large, the combined action of the sharp glass and the liquid served for their disintegration. The longer the shaking, the more perfect was the diffusion of the particles. It could not, however, be continued beyond a comparatively short period of time, because of the multiplication of the organisms. With the quantities of tissue above stated, ten minutes' shaking was selected as a happy medium between an undesirable multiplication of the organisms on the one hand and the retention of the organisms by the tissue, and the consequent lowering of the numbers found, on the other.

Still it is quite possible that by the method above described not all of the organisms are dislodged from the tissue. The fact that all of the experiments were conducted in exactly the same way, however, would render the results reported strictly comparable.

The suspension of organisms, prepared according to this method, was transferred by accurately graduated pipettes in portions of 1 cc, or more, to petri dishes and the desired nutrient material added. Such a procedure sufficed for the detection of aerobes and facultative organisms. For the anaerobes the double-plate method of Wright was adopted. This consists in the use of the cover of the petri dish as a holder for the medium and the organisms, and into it is set the lower part of the dish. If the dishes are flat, the cover is of fair depth, and the inner dish is lowered into the outer one in a slanting fashion, air bubbles will ordinarily be expelled, and there will be an air-free layer of nutrient media between two flat glass surfaces, enabling one to study or count readily the colonies which develop.

Since these plates are easily contaminated after sterilization, even in the short time required for cooling and in the transference during manipulation, a special metal container was obtained, in which they were sterilized and kept until the moment of inoculation.

There is generally a small space between the inner and the outer dish. This permits a drying out of the media, even after short periods of incubation, and makes the plates quite impracticable for longer periods. Therefore, the space between the two dishes was filled with a low melting paraffin, which had the additional advantage of excluding both air and dust.

The number of colonies developing on plates so prepared permits the enumeration of both facultative and anaerobic organisms, but for the separation of these two classes it would be necessary to transplant each colony or type of colony, submitting it to appropriate conditions to determine its relation to oxygen. An attempt was

made so to test each colony. It was soon found, however, that for the available laboratory force the number of subcultures was becoming unmanageable. Hence the search for anaerobes was restricted more especially to those organisms causing putrefaction of proteid in the sense of the German "Fäulnisprodukte,"[a] which organisms are generally of the spore-forming type, and which resist a temperature of 80° C. for ten minutes.

Accordingly, after plating the suspension for (1) aerobes and facultatives, and (2) anaerobes and facultatives, it was heated for ten minutes to 80° C., and again plated according to Wright's method. The colonies developing under these conditions were in such numbers that their further study was possible.

The chicken to be examined was placed on a clean metal dissecting tray. An incision was made in the skin along the entire length of the breast bone. With a small sharp knife, the fascia beneath was loosened and the integument laid back, exposing the pectoralis muscle. By the aid of sterile knives and scissors, a piece about one inch square was removed from the upper portion of the *Pectoralis major*. Care was exercised in the removal of this muscle not to contaminate the fibers of the *Pectoralis minor* lying beneath it.

As soon as the latter muscle was exposed several small pieces were quickly taken from it and placed in the Erlenmeyer flask holding broken glass. After reweighing, and so determining the weight of the muscle excised, the physiological salt solution was added and the flask shaken for ten minutes. Definite volumes of the physiological salt solution were then transferred to petri plates, the desired medium added, and the organisms evenly distributed by a rotary motion of the plate, which was placed in the temperature at which incubation was to take place. The period of this varied, of course, with the degree of heat. Those plates developing at 37° C. required but 2 or 3 days; those at 20° C. needed from three to seven days, while the plates grown in cold storage, at −1.67° C., did not show well developed colonies for several weeks, generally six to eight.

EXAMINATION OF FRESH AND COLD-STORED CHICKENS.

A study of the muscle from the breast, thigh, and walls of the abdominal cavity, tested according to the plan described, yielded sterile plates. Whether portions of flesh incubated in a nutrient fluid would give a bacterial growth has not been determined for all cases. Two experiments gave negative results.

As indicated in the following table, the cold-stored chickens have not been sterile. The thigh of No. 84 shows the greatest number

[a] Rettger, Further Studies on Putrefaction, J. Biol. Chem., 1908, 4: 45.

of organisms, namely 5,564, which developed at 20° C. For chicken No. 82, in storage 4 years, the maximum number of organisms is only 254, these colonies developing at 37.5° C. as well as at 20° C. In No. 85 the organisms were more plentiful in the muscles of the breast than in the thigh, which is not usually the case.

An attempt was made to divide the organisms into groups according to their behavior toward oxygen. Such a grouping must of necessity be somewhat crude, yet the figures which it furnishes are of interest when considered in connection with the chemical work already reported. Those organisms either demanding or tolerating an abundant supply of free oxygen are usually present in the greatest numbers. Occasionally the cultures which exclude obligate aerobes and permit the growth of anaerobes as well as facultatives show the greater number, while the organisms which are not killed by a temperature of 80° C. for ten minutes, and which can develop under anaerobic conditions, are usually greatly in the minority, or even entirely wanting. It is of interest to note that such bacteria in the chickens stored for 14 months are very few. The chickens stored for 2 years yield a larger representation and in the 4-year birds they form a considerable proportion of the total number. Another fact of interest is the relative prevalence of this class in the walls of the abdominal cavity.

All of the various types of colonies resistant to 80° C., and occurring under anaerobic conditions, were transferred and studied to determine whether they were obligate anaerobes or facultative organisms. Though many colonies have been tested, in not a single instance has an obligate anaerobe been found. No special tests have been applied to determine the presence of gas-forming organisms in the tissues, but in almost every case there has been used a lactose agar in some of the petri plates, poured according to Wright's method. Gas production under such conditions has not been observed.

The character of the flora is varied and, as far as a rather superficial study can determine, it belongs chiefly to the ordinary water and air occurring types. The large number of brilliant chromogens is striking; the percentage of motile organisms is high. Molds are at all times abundant and various species of Mucor, Penicillium, and Aspergillus have been noticed.

Bacterial content of cold-storage chicken muscle.

Description of sample.	Incubation temperature.	Aerobes and facultative organisms per gram.			Facultative and anaerobic organisms per gram.			Anaerobes and facultative organisms resistant to 80° C. per gram.		
		Inner thigh.	Breast.	Abdominal wall.	Inner thigh.	Breast.	Abdominal wall.	Inner thigh.	Breast.	Abdominal wall.
	° C.									
No. 4—storage 14 months	37.5	1,000	714	1,995	250	71	665	0	0	0
	20	5,564	1,501	1,995	1,824	714	1,164	0	0	0
	0 to −1.67	3,374	1,608	1,621	0	0	0	0	0	0
No. 85—storage 14 months	37.5	202	1,961	2,166	0	767	1,399	0	0	73
	20	606	5,202	3,321	303	3,752	1,660	0	0	0
	0 to −1.67	0	0	0	0	0	0	0	0	0
No. 79—storage 2 years	37.5	106	38	.....	160	0	.....	106	0	.....
	20	498	126	.....	106	50	.....	53	.....	.....
	0 to −1.67	0	0	.....	0	0	.....	0	0	.....
No. 82—storage 4 years	37.5	254	229	0	0	.....	77	0	0	77
	20	254	45	0	127	145	0	42	38	0
	0 to −1.67	51	76	0	0	0	0	0	0	0
No. 83—storage 4 years	37.5	55	246	150	442	35	253	55	0	126
	20	0	107	150	0	0	0	0	0	0
	0 to −1.67	0	0	0	0	0	0	0	0	0

HISTOLOGICAL STUDIES.

TECHNIQUE.[a]

A histological study was made of the muscle of the breast, using, generally, the *Pectoralis major*, and in a number of cases the study has been extended also to the muscle of the inner thigh and to the walls of the abdominal cavity.

The tissue was removed when making the bacteriological examination and fixed in alcohol, Zenker's fluid, and Orth's fluid. It was found, for practical purposes, that the latter reagent was greatly to be preferred, both for rapidity of fixing and the final cutting of thin sections. Both the Zenker and the alcohol caused the fibers to break and pull apart so that it is frequently an impossibility to obtain sections which are trustworthy, especially from chickens which have been in storage for a considerable length of time.

The small pieces of tissue remained in Orth's fluid for from 18 to 24 hours. They were then washed in running water for another 24 hours, and run through alcohols of increasing strengths until finally absolute alcohol was reached. From this they were put into anilin oil until clear, which operation requires only a few minutes and must be very carefully watched; then into xylol until all the anilin is extracted; next into a mixture of xylol and a paraffin melting at 52° C., which mixture is preferably kept at a temperature of about 37° C., and finally into a 52° C. melting point paraffin where, with several changes, 24 hours are needed for a complete infiltration.

[a] The histological preparations used in this study were made by E. Q. St. John.

It was found that the more rapid methods now much used, and which serve admirably for the study of many ordinary pathological and normal tissues, will not serve for the tracing of the changes sustained by the muscle during periods of cold storage.

The sections were cut as thin as possible—usually about 5 microns in thickness. The longer the fowl remains in storage, apparently, the more difficult it is to obtain thin sections of the tissue, there being a tendency to crumble, which makes it necessary to cut thicker sections, and even then they are exceedingly difficult to get.

A number of stains have been tried for the differentiation of the tissue elements. As a general routine stain Erlich's acid hæmatoxylin, followed by eosin, is very satisfactory. Not only does this differentiate the nuclei and cytoplasm, but it also stains bacteria. The color of the latter is that of the nucleus. Hence, unless the organisms are morphologically very distinct, or in such masses that their identification is comparatively simple, such staining will not serve as a method of differentiation, the long, slender nuclei of the muscle and of the connective tissue being readily confounded with bacilli.

After many trials of the ordinary routine methods of staining, most of which were distinctly unsatisfactory in various ways, Borrel's stain as modified by Coca [a] was finally adopted. This process as used consists of the following steps:

(1) Fixation in Orth's fluid.

(2) Paraffin sections.

(3) Removal of the paraffin by xylol, and of the xylol by 95 per cent alcohol.

(4) A concentrated aqueous solution of magenta red (Grüblers) for 30 minutes.

(5) Rinsing in water.

(6) Ten to fifteen minutes in indigo carmine (Grüblers) 0.75 gram, saturated aqueous picric acid 100 cc, and distilled water 100 cc.

(7) Differentiation in 95 per cent alcohol until the excess of the magenta red is removed, which may require 10 minutes and is indicated by the failure of the alcohol to color pink. Occasionally this treatment does not give a sufficient brilliancy to the nuclei, which should be a clear carmine red. When such is the case the slides are flooded for about 1 minute with magenta red, which is then quickly washed off with 95 per cent alcohol, just enough absolute alcohol following this for complete dehydration.

(8) Clearing in carbolic acid and xylol, as quickly as possible. If the dehydration with the absolute alcohol has been complete two floodings of the slide ought to suffice for the clearing.

(9) Mounting in xylol balsam.

This process can be carried through without any difficulty on the slide, and a number of sections can be stained and differentiated at the same time.

The muscle fibers are a brilliant green; connective tissue fibers are blue; the nuclei are clear carmine red, and bacteria are also red, but have usually much more of the purplish tinge than do the nuclei.

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[a] An Improved Method of Staining Fibroglia, Myoglia, Myo-fibrillæ of Striped Muscle, etc., U. of P. Medical Bulletin, June, 1907, p. 60.

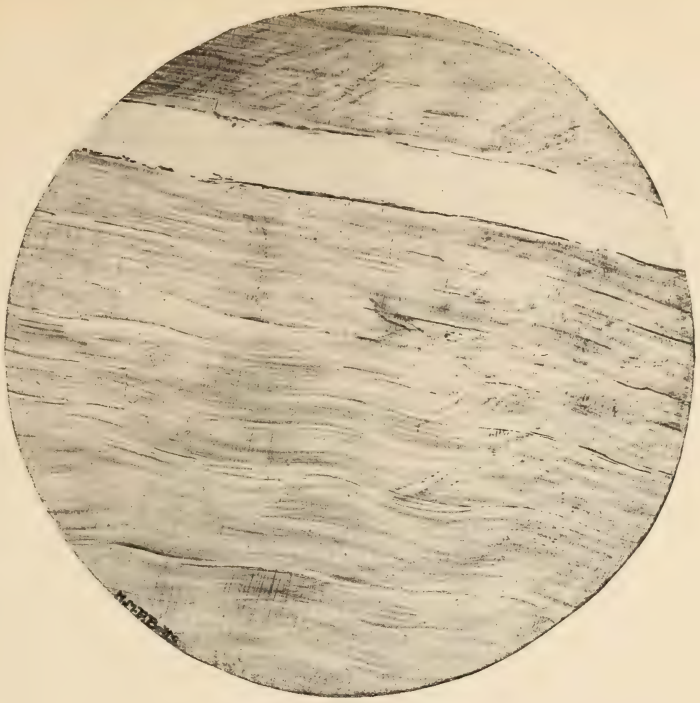

FIG. 1.—LONGITUDINAL SECTION.

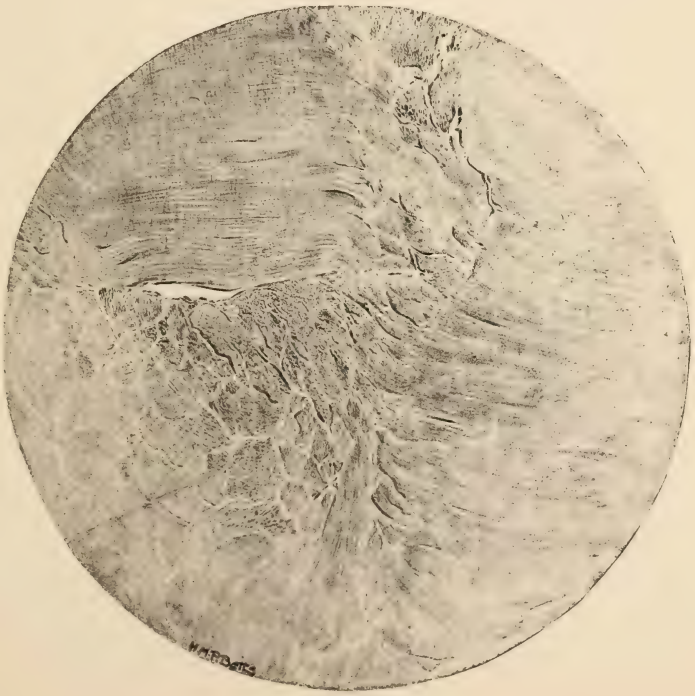

FIG. 2.—TRANSVERSE SECTION.

NORMAL CHICKEN MUSCLE.

[Zeiss: 4mm. objective, apochromatic; No. 6 ocular, compensating.]

EXAMINATION OF FRESH AND COLD-STORAGE CHICKEN MUSCLE.

The histological structure of fresh chicken muscle, as seen in the *Pectoralis major*, is shown in Plate VI. The fibers in the fresh muscle have a wavy outline, lie close together in bundles of six to ten, and often are so tightly adherent that their individuality is difficult to trace. The endomysium stains sharply and clings to the bundles. There is no evidence of a structureless, coagulable material between either the bundles or the individual fibers. While the cross markings are visible, they are not especially distinct, and appear as short segments instead of the clear, dark bands which text-book illustrations are apt to show.

The cross section of the muscle emphasizes the close packing of the fibers and the lack of material between them.

The examination of the longitudinal section of the breast muscle of chicken No. 84, which had been in storage 14 months, yields a picture varying greatly from that of the fresh tissue. The integrity of the small bundles is very largely obliterated, the individual fibers being pulled apart and in many places separated by wide spaces. Throughout this tissue lying between the bundles there is a markedly granular material, the origin of which can be traced to the muscle fiber itself, and is undoubtedly a product of its degeneration. This material has taken on a stringy, reticulated character, so much so that without careful study it might be confounded with a degenerated reticular tissue. It is constantly found, however, running back to the fiber itself, the structure of which becomes gradually more distinct until finally the cross markings and general outline of it are unmistakable.

In the muscle of this tissue there is also a tendency to a longitudinal gathering of the fibrils into masses sometimes one-third or one-quarter the size of the original fiber, with a space between the bundles, and the whole still surrounded by the sarcolemma. While the waviness of the normal muscle and the appearance of contractility are entirely lost in these muscles kept in cold storage, there is, however, a tendency to a twisting of the muscle fiber, making "knees," as they may be designated, along its length and giving it a very irregular appearance. With the longitudinal splitting there is also a marked tendency to a transverse fracture, in many places the fibers being split into short fragments. (Plate VII, fig. 1.)

The cross section of the muscle illustrates well a number of the points which were observed in the longitudinal section. It shows, for instance, the breaking of the small bundles by many transverse lines, which are probably the indications of the bundles of fibrils before noted. The reticulated structure is seen between the muscle bundles

with great distinctness, and its very large quantity is often more apparent here than in the longitudinal section. The connective tissue fibers have lost much of their fibril quality, showing a tendency to swell and in some cases giving almost an appearance of a mucoid degeneration. (Plate VII, fig. 2.)

The staining character of this cold-stored muscle fiber shows considerable inequality. With the Coca modification of the Borrel stain the fiber of the normal muscle is a perfectly even, clear green. The fiber of the muscle under discussion is apt to stain irregularly, and there is a tendency to a brownish yellow tinge in many places rather than the clear green. The nuclei still take the crimson red. The exuded substance from the muscle fiber takes a green which is almost exactly that of the fiber itself. The connective tissue, which is normally a very brilliant dark blue, has lost a good deal of its color and has become a dirty green. It is quite possible to trace these color changes from tissue which is normal, through the various gradations of color and texture, until finally the extreme of the alteration is reached.

Though the bacteriological examination of the muscle of these chickens shows a considerable number of organisms in the breast tissue, organisms could not be found in the sections by microscopic study. When the size of the breast muscle of a chicken is considered as compared with the exceedingly small area represented by even a large number of sections, it must be looked upon more as good luck than good management when there is success in locating bacteria microscopically in such a tissue, and the fact that none were found in the muscle of the breast is by no means conclusive proof of their absence.

In the muscles of the inner thigh it was possible to demonstrate under the microscope a very distinct bacterial invasion. This is represented in Plate VIII, which shows the longitudinal and the cross sections of a muscle fiber that has been penetrated by organisms, a colony of which has developed to such size that the fiber itself is a mere shell. With a magnification of 400 or 500 diameters these tightly packed organisms appear, as in the drawing, as almost a solid mass, staining from a dirty green to a deep purple. With a magnification of 1,000 diameters, or over, it is possible on the edges and in the thinner portions of this mass to resolve it into individual organisms which, in this case, are morphologically rod forms.

There will be noticed in the cross section a more brilliant purple than prevails in the longitudinal section, and in the longitudinal section, also, the more distinctly purple tone fades gradually until it is lost in a dirty green. Such variation seems to be due to the progress of the organism in the fiber, those portions which are but slightly invaded retaining the green color and losing it little by little until,

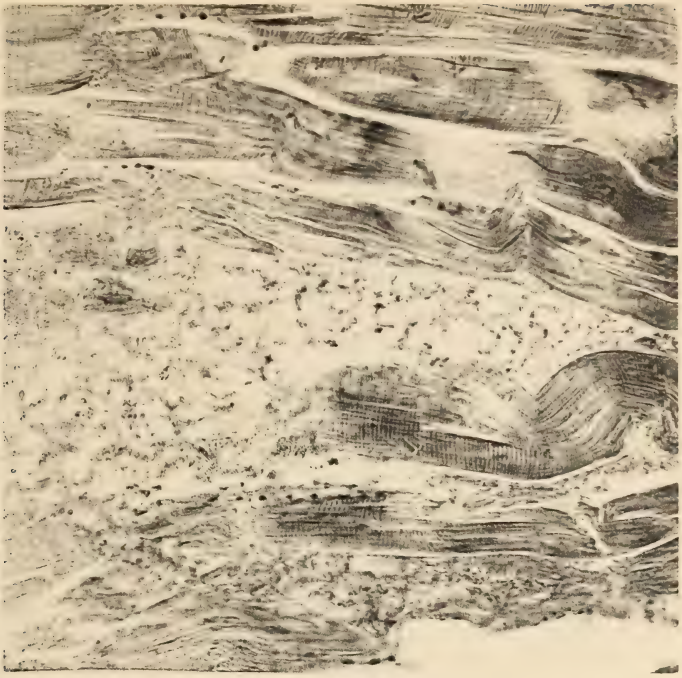

FIG. 1.—LONGITUDINAL SECTION.

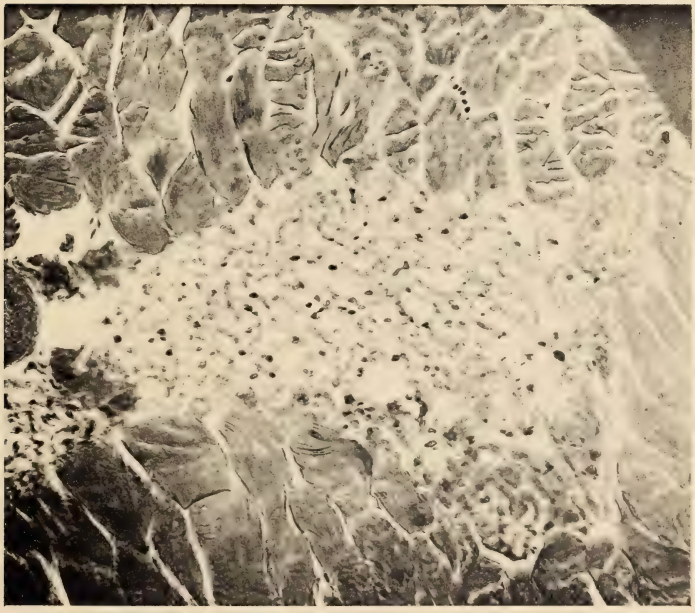

FIG. 2—TRANSVERSE SECTION.

BREAST MUSCLE OF CHICKEN IN COLD STORAGE 14 MONTHS.

[Zeiss: 4mm. objective, apochromatic; No. 8 ocular, compensating.]

FIG. 1.—LONGITUDINAL SECTION.

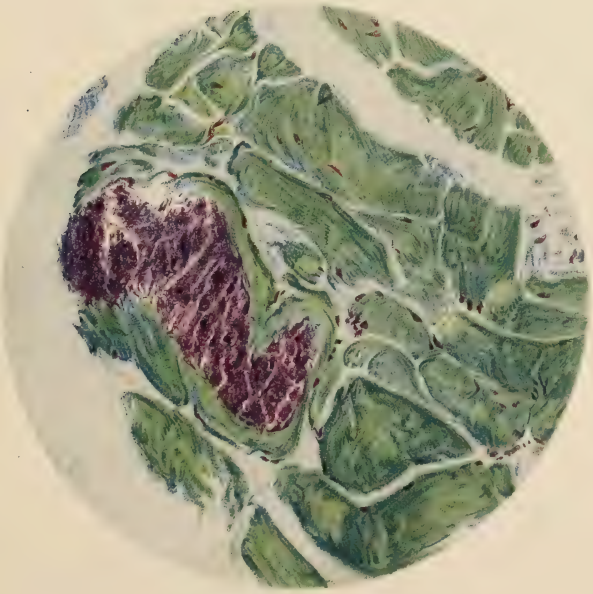

FIG. 2.—TRANSVERSE SECTION.

THIGH MUSCLE FROM CHICKEN IN COLD STORAGE 14 MONTHS,

SHOWING INVASION OF FIBER BY BACTERIA.

[Zeiss: 4mm. objective, apochromatic; No. 6, ocular, compensating.]

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when the mass of organisms has quite obliterated the tissue, the color is deep purple.

Such changes have been traced in a number of fibers in this leg muscle. It has also been possible, in chickens kept at ordinary temperatures until decomposition had undoubtedly set in, to locate the same phenomena not only in the muscles of the leg but in other muscles also.

The chickens No. 69 and No. 79, which had been kept in storage for 2 years with two thawings and one refreezing, furnish an exceedingly interesting study. The degeneration here has progressed much further than that observed in the chickens in storage 14 months.

In chicken No. 69, also, a study has been made of the tissue thawed by soaking and thawed by cold air. Microscopic differences between these two methods of thawing are exceedingly marked. The longitudinal section of the chicken which has not been soaked shows fibers smaller than are the normal, glassy, smooth, with brilliant cross markings, blotchy staining, and sharply refractive outlines. Between these tightly bound bundles are spaces sometimes considerably wider than the bundles themselves and filled with a homogeneous, granular material which stains light dirty green and which gives the appearance of a finely divided amorphous precipitate. These masses are the exudate from the degenerated fibers, and a very large part of almost any field that one may select consists of such material.

The whole tissue, even though very thinly cut, has a dense appearance, and the usual translucency is much decreased. (Plate IX, fig. 1.)

The cross section of this tissue makes even more manifest the large spaces devoted to the exuded degenerated substance. While the individual fibers are, in most cases, distinct, the spaces between them are small, so that the bundles themselves are compact but lie widely separated by the exudate. The staining is irregular, the fibrillæ themselves, which are seen plainly on the cross section of fresh muscle, are almost entirely invisible, a smooth glassy appearance now being prevalent in the transversely cut fiber. (Plate IX, fig. 2.)

The muscle which has been thawed by soaking shows, in longitudinal section, a fiber almost twice the width of that which is unsoaked, and the large masses of amorphous material are now reduced to mere remnants which lie as a clear green mass of not very tightly packed granules between the bundles of fibers. In many cases it is difficult to find even these remnants and, while the individual fibers are not tightly packed into small bundles as they are in the fresh tissue, they are separated by only small spaces.

The uneven staining of the fiber itself still holds and is more marked than in the unsoaked portion of muscle. The tissue, as seen under

the microscope, has an exceedingly blotchy appearance, in some places showing clear yellow or orange-yellow segments in a fiber which is only banded with green. The cross markings are sharp and clear.

The longitudinal separation of the fibrillæ is still very prominent, but even more marked is the tendency of the muscle to break into short lengths, or, if not absolutely to break apart, to show bands of amorphous material which apparently lie inside of the sarcolemma and which have lost all semblance to characteristic striated muscle tissue. (Plate X, fig. 1.)

The cross section of the soaked muscle is quite different from the cross section of the unsoaked. The fibers are distributed evenly throughout the whole field. Small spaces indicate the endomysium, and here and there is a space holding a few granules which correspond to the great mass of amorphous material that characterizes the unsoaked muscle. The internal structure of the fiber itself has not apparently undergone any marked change by the soaking process. It is still much smoother than the normal. In the cross section, even more markedly than in the longitudinal, is seen the tendency to uneven staining, certain fibrils showing orange red instead of green, others yellow, and others giving clear green. (Plate X, fig. 2.)

In the muscle of the chicken in storage 4 years all of the forms of degeneration before mentioned are not only visible but more pronounced. The exuded material, however, has taken on a vacuolated appearance, and in certain places this form of degeneration can be seen overspreading a number of fibers, the edges of which gradually lose their outline until all the fibers become merged into a solid mass of a dense greenish-blue staining vacuolated substance. This would seem to be a much exaggerated extension of the vacuolated bands which were noted in the chicken in storage for 2 years, but which had not at that time ruptured the sarcolemma. (Plate XI, fig. 1.)

Very few of the fibers in the tissue kept for 4 years show the characteristic green staining. They are a dirty yellow green instead, with here and there a clear green staining mass in the fiber itself. The cross markings are still visible on most of the fibers and, where they do exist, are exceedingly brilliant. The brittleness of the fiber is very apparent, not only showing itself in the difficulty experienced in cutting thin sections but also indicated by the many transverse and longitudinal cracks which break the individual fibers in every direction. (Plate XI, fig. 2.)

Attention has been called in a previous section of this report to the macroscopic appearance of the intestines of chickens kept in cold storage. The microscopic study of the character and sequence of these changes in the market chickens discussed here has not been made, but scattering examinations have shown that the macroscop-

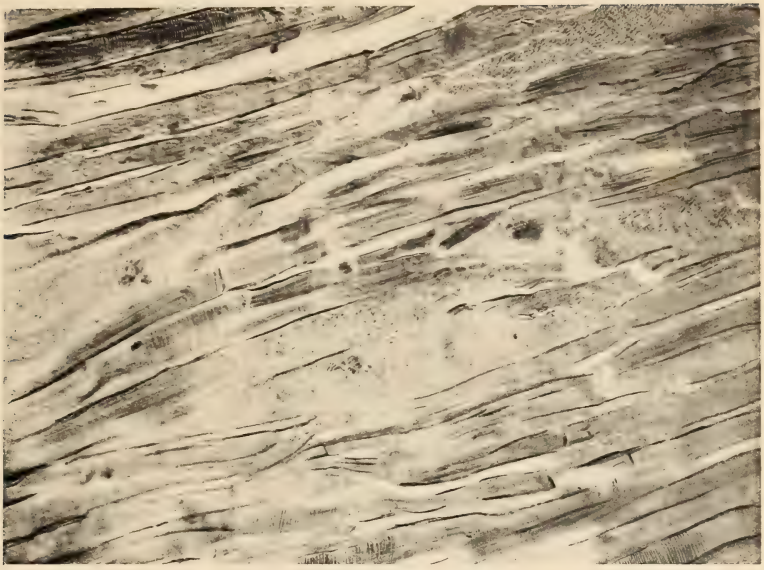

FIG. 1.—LONGITUDINAL SECTION.

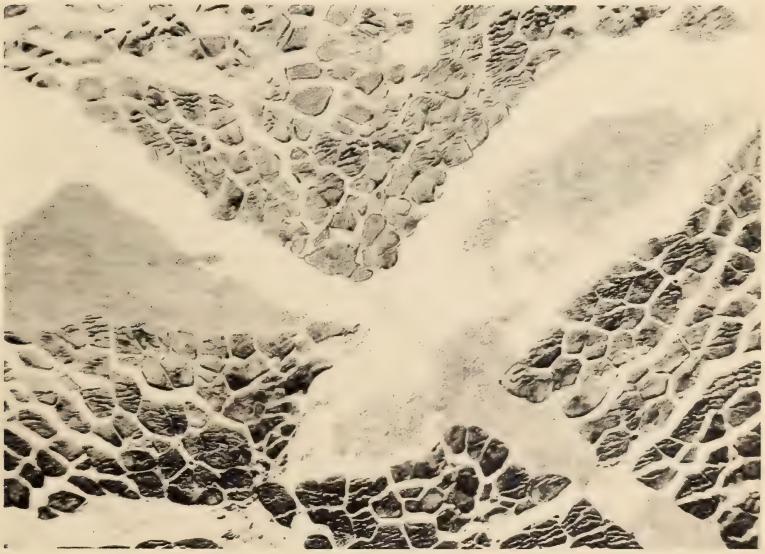

FIG. 2.—TRANSVERSE SECTION.

UNSOAKED BREAST MUSCLE OF CHICKEN IN COLD STORAGE 2 YEARS.

[Zeiss: 16mm. objective, apochromatic; No. 12 ocular, compensating.]

FIG. 1.—LONGITUDINAL SECTION.

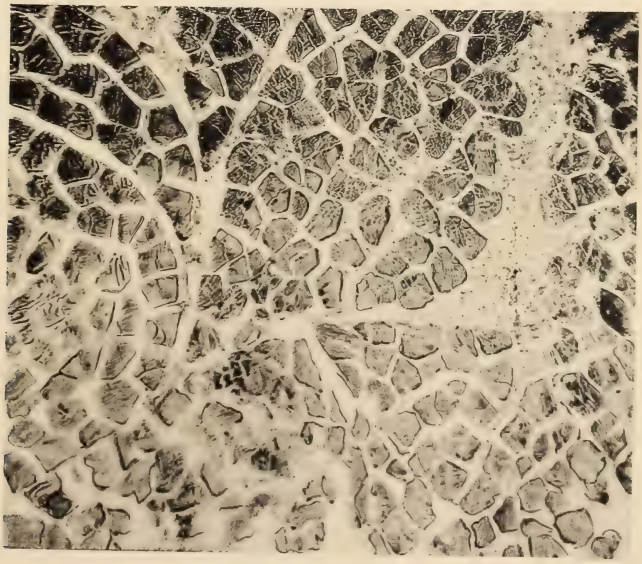

FIG. 2.—TRANSVERSE SECTION.

SOAKED BREAST MUSCLE OF CHICKEN IN COLD STORAGE 2 YEARS.

[Zeiss: 16mm. objective, apochromatic; No. 12 ocular, compensating.]

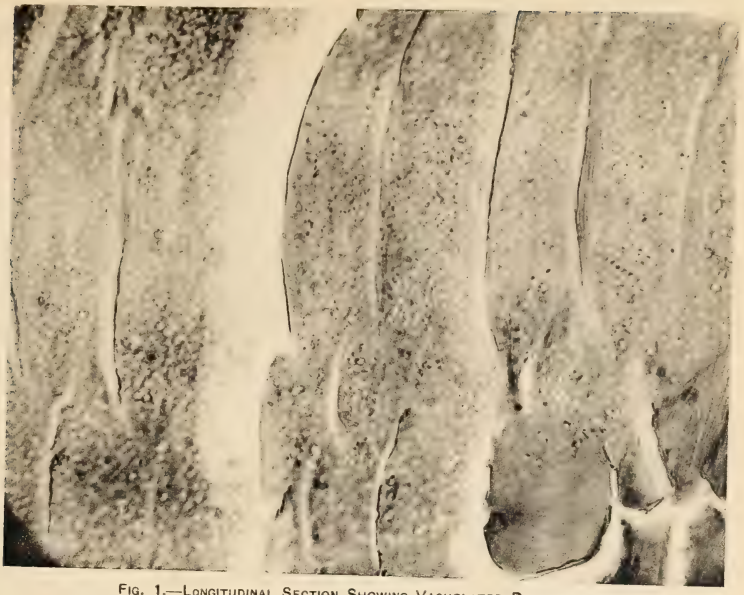

FIG. 1.—LONGITUDINAL SECTION SHOWING VACUOLATED DEGENERATION.
[Zeiss: 4mm. objective, apochromatic; No. 8 ocular, compensating.]

FIG 2.—LONGITUDINAL SECTION SHOWING VARIED AND EXTENSIVE DEGENERATION.
BREAST MUSCLE OF CHICKEN IN COLD STORAGE 4 YEARS.
[Zeiss: 16mm. objective, apochromatic; No. 12 ocular, compensating.]

ical appearance is more than explained by the structure of the tissue as revealed under the microscope.

Plates XII and XIII show the character of the intestine of a fresh chicken and also of a chicken in cold storage for six months. It will be observed that the muscular coats of the cold-stored chickens have quite lost their characteristic structure. Apparently they consist almost exclusively of the sheath of the fibers which, if they contain muscle substance in any quantity, have it so altered chemically that standard muscle-staining dyes do not affect it. What remains of the individual fibers is a mass, loosely put together, which would offer but little resistance to the migration of bacteria.

So far as the cellular portions of the intestine are concerned they are reduced to a minimum. Either the intestines are obliterated or the cells are so dried and disintegrated that they are mere fragments. Though the cell, as a whole, is in bad condition, the nucleus has apparently offered greater resistance to the corroding action than has the cytoplasm of the cell.

GENERAL DISCUSSION.

Bacteria and enzymes are most directly concerned in the question of flesh decomposition, the effect of air, light, temperature, humidity, and such conditions as are ordinarily considered responsible for alterations in organized tissues being in reality factors influencing the growth or activity of one or the other, or both, of the agencies stated. The investigations of flesh decomposition which have been reported have been conducted usually at room temperatures or at body heat. Such studies at temperatures near 0° C. are almost entirely wanting, probably because of the general idea that enzymic and bacterial activities cease at or about zero.

RESISTANCE OF BACTERIA TO LOW TEMPERATURES.

The resistance of bacteria to low temperatures is well known, and a number of investigators have subjected various species of organisms to almost the lowest limit of modern cold. In 1884 Pictet and Young [a] exposed *B. anthracis*, *B. subtilis*, *Micrococcus leuteus*, the bacillus of symptomatic anthrax, beer yeasts, and smallpox vaccine to temperatures varying from −70° C. to −130° C. and for periods of from 20 to 108 hours. The bacteria proper were not destroyed; the yeasts were intact microscopically but their functions were lost, as was also the activity of the smallpox vaccine. Macfayden [b] exposed a number of bacteria, both benign and pathogenic, to the temperature of liquid air (−190° C.), first for a period of 24 hours and

[a] Compt. rend., Jan. to June, 1884, p. 747.
[b] Lancet, 1900, *1* [849]: 1130.

then for 7 days. Buchner's zymase was also tested and survived for 20 hours. Some photogenic bacteria exposed to this extreme cold promptly ceased to emit light, but thawing soon caused a resumption of activities.

Macfayden says: "It is a remarkable fact that, notwithstanding the enormous mechanical strain to which the organisms must have been exposed, a strain far exceeding in amount any capable of being produced hitherto by direct mechanical means, not the slightest structural alteration could be detected." There was no impairment, according to the observations of this author, in the vitality of the organisms. Belli [a] has also subjected the bacteria of chicken cholera and anthrax to the action of the temperature of liquid air, without altering their properties.

DEVELOPMENT OF BACTERIA AT LOW TEMPERATURES.

At temperatures approaching the zero point not only viability but fertility has been proven, and the literature shows that quite a number of investigations have been conducted, and the field is wider than would at first sight appear. Forster,[b] for example, finds that certain photogenic bacteria grow at 0° C. and that organisms capable of developing at this temperature are to be found in earth, milk, flesh, spring water, etc. He also states that the plague bacillus is capable of growing at zero. He determines, too, the rate of growth in an ice calorimeter, and finds that from 10 to 12 days are usually sufficient. Discussing the question of bacterial growth in the German "Kühlhaus," Forster[c] concludes that the changes induced are slower than at ordinary temperatures, but are not stopped nor essentially altered. S. Schmidt-Nielsen[d] found five species of bacteria which developed at zero in the Strassburg water supply, from 10 to 40 days being required for appreciable growth. He found, also, an actinomyces which required 80 days for development.

These organisms are *Bact. fluorescens nonliquefaciens, B. granulosum, B. paracoli gasoformans anindolicum, B. tarde fluorescens,* and *B. radiatum.* Fischer[e] and Havemann[f] have also studied organisms which are capable of making a growth at zero, the former isolating fourteen species from various sources and the latter obtaining

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[a] Riforma Medica, 1901.

[b] Über einige Eigenschaften leuchtender Bakterien, Zentrbl. Bakt. Paras., 1892, *12*: 431.

[c] Über die Entwickelung von Bakterien bei niederen Temperaturen, ibid., p. 431.

[d] Über das Vorkommen psychrophiler Bakterien, Zentrbl. Bakt. Paras., 1902, *9*: 145.

[e] Bakterien Wachstum bei 0°, etc., Zentrbl. Bakt. Paras., *4*: 89.

[f] Inaug. Diss. Rostock, 1894, Über das Wachstum von Mikro-organismen bei Eisschranktemperatur.

SMALL INTESTINE OF FRESH CHICKEN.
[Zeiss: 4mm. objective, apochromatic; No. 6 ocular, compensating.]

SMALL INTESTINE OF CHICKEN SIX MONTHS IN COLD STORAGE.

[Zeiss: 4mm. objective, apochromatic; No. 6 ocular, compensating.]

some from sewage. Müller [a] has isolated 36 species of fungi that will grow at 0° C. from such sources as flesh of fish and cattle, intestinal contents of fish, vegetables, grain, and garden earth. Ten of them were found in flesh and nine in earth.

Not only are such organisms widely distributed, but very pronounced results in nature have been credited to them. Schmelk [b] believes the green color of the Norwegian glaciers to be due to the presence of *B. fluorescens liquefaciens*, an organism which he finds in such ice, and which develops rapidly at zero. Glage [c] ascribes considerable importance in the maturation of meat to what he terms "aroma-producing bacteria," organisms which develop in the German "Kühlhaus," where the temperature is usually from +2 to +5° C., and which he finds infecting not only the rooms, but the carts, trucks, and, in fact, the entire slaughterhouse. Their growth at +2° C. is rapid; at 37° C. it is very slow.

That naturally occurring bacteria will develop in milk kept at −1.67° C., with a fair degree of rapidity and in numbers which finally reach several billions per cubic centimeter, has been demonstrated by Pennington.[d] Certain species of organisms were found to outrun others so decidedly that they were finally present in almost pure culture. Among such were most commonly found *B. solitarius* Ravenel, *Bact. aerogenes*, *B. coli*, and *B. formosus*. However, a number of other species also grew below 0° C., as indicated by plates made and incubated at the temperature at which the milk was kept, namely, −1.67° C. These bacteria developed in spite of the fact that the milk was a semisolid mass of crystals, so solid, indeed, that it had to be dipped out of the container.

A multiplication of organisms in ice cream when kept in a frozen condition, either by packing in ice-salt mixture, which gives a temperature of about −17° C., or in a cold-storage warehouse where the temperature varied from −18° to −23° C., is reported by Wiley.[e] Apparently there is a series of curves of growth, varying both in periods of time and in intensity for different samples, and, in all probability, depending on the rise and development of a succession of species.

It is commonly stated that pathogenic organisms do not develop at zero, and, broadly speaking, such is usually the case. That it can not be applied as a sweeping assertion is indicated by the statement of Forster, previously cited, that the bacillus of the plague does grow at

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[a] Arch. Hyg., 1903, *47*: 127–193.

[b] Eine Gletscherbakterie, Zentrbl. Bakt. Paras., *4*: 545.

[c] Zts. Fleisch- Milchhygiene, 1900–1901, p. 131.

[d] Bacterial Growth and Chemical Changes in Milk Kept at Low Temperatures, J. Biol. Chem., 1908, *4*: 353.

[e] Milk and Its Relation to the Public Health, by various authors, Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service, 1908, Bul. 41, p. 257.

zero. Conradi and Vogt[a] have found that *B. proteus fluorescens* will grow at zero, and B. Fischer[b] found a pus-forming coma bacillus which flourishes at this temperature.

INHIBITORY ACTION OF LOW TEMPERATURES ON BACTERIA.

The reverse of the question of life and reproduction at low temperatures may be found in those investigations dealing with the destruction or inhibition of bacteria by cold. Repeated attempts to induce certain pathogenic organisms to grow at temperatures even somewhat above zero have failed, as did those of Brehme,[c] who worked on cholera and typhoid, and of Dieudonne,[d] who sought through successive lowering of the temperature by 5° stages to cultivate an anthrax bacillus resistant to cold; +10° C., however, was the limit of growth for this organism.

In relation to the self-purification of ice from bacteria Prudden[e] was one of the earliest workers. He concluded that 51 days were sufficient to kill *B. prodigiosus; Stap. pyogenes aureus* was reduced to one-fifth of its original number in 66 days, and *B. typhosus* was greatly reduced in 103 days. Alternate freezing and thawing is more effective as a killing agent than a single freezing of longer duration. Prudden states that the act of freezing itself either kills or reduces vitality, an opinion in which other investigators, Macfayden, for example, do not concur. Undoubtedly, however, according to the work of Sedgwick and Winslow,[f] continued effects of freezing temperatures, at least so far as organisms in water are concerned, tend to greatly reduce their numbers or even to kill them outright. These authors, however, lay particular stress on the time factor, believing that ice from polluted streams should be stored for months to permit the cold to thoroughly act on the organisms present.

Smith and Swingle[g] believe that the effect of very low temperatures is overestimated and that the cold produced by ice and salt mixture, about −17° C., is as efficacious as liquid air. They hold that the critical point for the generality of organisms lies at or near 0° C., but

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[a] Ein Beitrag zur Ätiologie der Weil'schen Krankheit, Zts. Hyg. Infekt., *30*: 287.

[b] Deutsche med. Woch., 1893, No. 25.

[c] Über die Widerstandsfähigkeit der Cholera Vibrionen und Typhusbacillen gegen niedere Temperaturen, Archiv. Hyg., *40*: 320.

[d] Beiträge zur Kenntnis der Anpassungsfähigkeit der Bakterien an ursprünglich ungünstige Temperaturverhältnisse, Arb. aus dem Kaiserl. Gesundheitsamte, *9*: 492.

[e] Bacteria in Ice and their Relations to Disease, with Special Reference to the Ice Supply of New York City, Medical Record, 1887, *31*: 341.

[f] Experiments on the Effect of Freezing and Other Low Temperatures upon the Vitality of the Bacillus of Typhoid Fever, with Considerations Regarding Ice as a Vehicle of Infectious Disease, Mem. Amer. Acad. Arts and Sci., 1902, *12* (5).

[g] Der Einfluss des Gefrieren auf Bakterien, Zentrbl. Bakt., I Abt. Ref., 1905–6, *37*: 357.

single individuals may withstand unfavorable conditions. Repeated freezing and thawing will completely exterminate some varieties, certain individuals of which will survive a single freezing and thawing. The interesting observation is also made that spore formers are more resistant than are vegetative cells.

From the foregoing summary it would appear that a considerable number of bacteria can, when the food supply is favorable, reproduce at 0° C. or even below that temperature, though growth is greatly retarded, and that freezing, even though prolonged, does not of necessity indicate sterility.

CHEMICAL CHANGES PRODUCED BY BACTERIA AT LOW TEMPERATURES.

For the purposes of this investigation the proof of the viability or even of the reproduction of bacteria at low temperatures does not suffice, since the changes taking place in certain foodstuffs kept in a frozen condition are under consideration, which changes are fundamentally chemical. It becomes necessary, then, to investigate the ability of bacteria, when maintained at low temperatures, to cause changes in such compounds as proteins, carbohydrates, and fats, it being commonly held that even if organisms should multiply under such conditions the results of their life processes would not correspond with those taking place at the optimum growth temperature.

Müller[a] has studied the evolution of carbon dioxid, hydrogen sulphid, the breaking down of protein, and such chemical changes as commonly accompany the growth of bacteria when the germs were maintained at a temperature of zero. He finds that for organisms growing at zero such chemical indications of activity are not wanting, but that the formation of the various decomposition products is greatly retarded and their production is very slow. Such results are quite in accord with another observation of Müller's, namely, that the life cycle of one of the organisms isolated, *B. fluorescens liquefaciens*, at 0° C., is twenty-four times as long as is the cycle at 25° C.

EFFECT OF LOW TEMPERATURES ON ENZYMES.

On this most important phase of bacterial growth and development there is almost a total lack of recorded observations, those changes which have been traced chemically in flesh foods chilled or frozen being ascribed to enzyme action exclusively. Undoubtedly enzymes play an important rôle in these decompositions, but it is quite possible that they are not the sole agency. Fermentation changes due to bacteria

[a] Über das Wachstum und die Lebenstätigkeit von Bakterien, sowie den Ablauf fermentativer Prozesse bei niederer Temperatur unter spezieller Berücksichtigung des Fleisches als Nahrungsmittel, Archiv. Hyg., 1903, *47*: 127.

in milk kept at −1.6° C. have been observed by Pennington.[a] In these experiments the acidity of the milk, which is due to bacterial activity, increased enormously. There was also observed a very pronounced hydrolysis of the casein, but such a degradation might be ascribed either to the natural enzymes of the milk or to those produced by bacteria or to a combination of the two factors.

That enzymic activity is not stopped at 0° C. or even below it seems to be generally accepted, and neither are the enzymes destroyed by cold. Macfayden[a] found that Buchner's zymase withstood for 24 hours the temperature of liquid air. Very recently Kovehoff[b] found that freezing for 24 hours did not destroy the proteolytic enzyme in wheat, peas, and the tissue of *Vicia faba*. After thawing, the enzyme caused a decrease in protein nitrogen, the nonprotein nitrogen in one experiment reaching 48.7 per cent.

Though able to survive low temperatures, the common enzymes are greatly hindered, and the usual course of action of some of them is so altered that their characteristic expression of it is quite lost. For instance, Müller,[a] experimenting with the action of rennin on milk, found that curding did not take place at 0° C., but, on the other hand, the enzyme still functioned to some extent, because, when brought to 40° C., the curding time was greatly accelerated. The following table illustrates this point:

5 cc milk at 0° C. + 1 cc of rennin, kept at 0° C. for—	Coagulation at 40° C. after—	
	Minutes.	Seconds.
60 minutes	2	00
75 minutes	1	50
90 minutes	1	45
105 minutes	1	10
120 minutes		55
150 minutes		45
24 hours		45
48 hours		45
96 hours		50

After 96 hours, at 0° C., the coagulation occurred as a fine flocking which made its recognition difficult. If other enzymes behave similarly it would tend to throw light on many obscure flesh decompositions which take place after cold storage has been maintained for long periods.

Some milk enzymes, however, are not only partly but wholly able to function, as is indicated by the fact that galactase acts in the ripening of cheese at low temperatures, and also Jansen's statement that this enzyme does not act more rapidly at 40° C. than at ordinary temperatures.

The optimum temperature for the action of the common enzymes lies not far from 40° C. However, it is not yet definitely proven that,

[a] Loc. cit. [b] Ber. d. botan. Ges., *25*: 473.

for certain animals, digestion does not proceed better at temperatures much below this. Müller [a] compared the action of pepsin from the stomach of the pike at 0° and at 24° C. and found that it took seven times as long to accomplish a given amount of work at the former as at the latter temperature, the pepsin being very active at 24° C.

Flaum,[b] working on the ordinary functions of the stomach, states that undoubtedly activity does not cease at zero. Fick and Murisier [c] state that the gastric juice of the pike can digest proteid at 0° C., and Hoppe-Seyler,[d] who studied the same fish, concluded that not only did digestion proceed at zero, but it was most rapid at 20° C., and quite active at 15° C. Krukenberg,[e] on the other hand, finds just the opposite—that 40° C. is the more favorable temperature, and his work is confirmed by Luchhau.[f]

Apparently the resistance to cold is not limited to proteolytic enzymes, since Kastle and Loevenhart [g] have noted that lipase, though splitting fat most rapidly at 40° C., is still active at −10° C., but its action is much slower. At 40° C., for example, 11.29 per cent of fat was hydrolysed, while at −10° C. only 0.70 per cent was changed.

THE ACTION ON FLESH OF BACTERIA AND ENZYMES AT LOW TEMPERATURES.

Review of the Literature.

A review of the chemical, bacteriological, and histological data recorded in connection with the analysis of the market cold-storage chickens made in this laboratory indicates that undoubtedly a certain amount of chemical change, and depending upon it certain histological alterations, go on at temperatures far below the freezing point. They have proven, also, that even four years at such low temperatures are not sufficient to render the flesh of chickens germ-free, and, judging from the work which has already been reported by various investigators, it seems probable that both bacteria and enzymes will resist intense cold for long periods, only thawing and a rise in temperature being needed to cause a rapid resumption of activities.

Some recent contributions to our knowledge of bacterial life at temperatures below zero would indicate that, if the organisms are pro-

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[a] Loc. cit.

[b] Über den Einfluss niedriger Temperaturen auf die Funktionen des Magens, Zts. Biol., *18* (Neue Folge, *10*): 433.

[c] Verhandlungen der Würzburger physiol. med. Gesell., 1872, N. F. *2*: 122.

[d] Pflüger's Archiv gesam. Physiol., *14*: 395.

[e] Versuche zur vergleichenden Physiologie der Verdauung mit besonderer Berücksichtigung der Verhältnisse bei den Fischen, Untersuch. aus dem physiol. Inst. zu Heidelberg, *2*: 395.

[f] Über die Magen- und Darmverdauung bei einigen Fischen, Inaug. Diss. Königsberg, 1878.

[g] Amer. Chem. J., 1900, *24*: 491.

vided with their natural environment, they will multiply when the medium is in a frozen condition. The experimental work which has been done in the past has used chiefly the usual laboratory media and pure cultures of laboratory-grown bacteria. It is perfectly possible that such conditions, since they are not comparable with those naturally prevailing, will lead to some erroneous results, and in view of recently demonstrated facts it becomes necessary to attack the problem of bacterial development in flesh foods from this point of view before the assumption can be accepted that temperatures below freezing guarantee a freedom from bacterial activity.

Observations on the growth of bacteria under the conditions of the very low temperature cold-storage houses, such as almost universally prevail in the United States, are entirely lacking. What information is to be had on the growth of bacteria in flesh when cold-stored comes from abroad, chiefly from Germany, where the temperature of the "Kühlhaus" rarely reaches 0° C. and is commonly several degrees above it. Exposed to such temperatures there is a unanimity of opinion regarding the "ripening" of the flesh, and the tenderness and flavor acquired in the course of it. To what this maturation is due, however, is not so well settled. Glage [a] would ascribe much of the flavor to "aroma-producing" bacteria which develop best at low temperatures; Müller,[b] on the other hand, believes that the process is essentially dependent upon the enzymes of the flesh itself. He holds that the temperature of the chilling room prevents putrefaction, and, therefore, all those poisonous properties dependent upon putrefaction, while it assists natural autolysis. Neither does he consider [c] that the changes in the ripening of meat are the early stages of putrefaction.

The observations made under commercial conditions he has reinforced by a study of freshly killed, bichlorid-washed fish, which was kept at 0° C. After 5 days there was an unpleasant taste and a characteristic odor, both of which appeared in 2 days when kept at 12° C. Schmidt-Nielsens kept a carp packed in ice for 14 days, and, though bacteria-free, it had, at the expiration of the above period, so unpleasant a taste that it was unfit for food. Müller determined the amount of nitrogen soluble in water in both mammalian and fish muscle kept at 0° C., concluding therefrom that an autolysis proceeds. He found that muscle loses its elasticity, becomes tender, and the clear red color changes to an opaque, dark red. The odor after 3 days at 25° C. or at 0° C. after 14 days is strongly acid.

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[a] Zts. Fleisch- Milchhygiene, 1900-1901, p. 131.

[b] Der Reifungsprozess des Fleisches. Zts. Fleisch- Milchhygiene, 1904, *14*: 217 and 337.

[c] Archiv Hyg., 1903, *47*: 127.

In 1897 Gautier[a] published the account of a chemical and histological examination of frozen flesh, chiefly of that sent to France in a frozen condition from South America and the United States. He stated that the flavor of the frozen muscle was never quite as good as the fresh. That the juice when exuded was always more abundant than from the fresh and that it contained globulins, albumins, peptones, and organic and inorganic constitutents. Among the most recent chemical studies of such flesh is that made by Rideal,[b] who determines the ratio of nitrogen to the total solids in both chilled and fresh meats and, finding them the same, excludes decomposition. Artificial digestion gave identical results with frozen and fresh muscle. He states that there were no signs of incipient decomposition. Neither bacteriological nor histological examinations were reported. However, this statement is made: "The tenderness of meat which has been frozen has been attributed to the slow action of sarcolactic acid, and the loosening of the intermuscular tissue promotes rapid decomposition."

Martel,[c] in a recent article on the cold storage of foodstuffs, favors cold rooms rather than a temperature sufficiently low to freeze, believing the latter only necessary when transportation for long distances is to follow. He states that bacteria do not readily penetrate the muscle, about 10 days being required to carry them within 1 cm of the surface, but that there is a marked autodigestion of the cell contents, due to the action of the cell ferments, which proceeds easily at 2° to 3° C., though below zero such action is stopped. As to the quality of the meat after storage he believes it to have an improved flavor if kept at 2° to 3° C. and not allowed to freeze. The cause of this improved flavor is ascribed to the aroma bacteria, which he considers desirable.

Accompanying the change in taste there are, according to Martel, alterations in the muscle fiber which consist in a change from translucent to opaque and from brilliant to dull, as well as from tough to tender. The reaction of the fresh muscle is neutral, but becomes acid as coagulation proceeds. In cold storage at 2° to 3° C. about 8 hours are required for a strong acidity to develop, and an odor, aromatic but not at all putrefactive, makes its appearance at the same time. When muscle is fresh it is very difficult to extract any fluid. After 3 days in storage flesh yields much juice. Microscopic examination shows an annihilation of the striations and abundant granulations.

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[a] Les viandes fraiches et congelées.

[b] Cold Storage, 1907.

[c] Conservation et maturation des viandes emploi du froid industriel, L'Hygiene de la viande et du lait, Vol. I, Nos. 1 and 2.

DISCUSSION OF RESULTS OBTAINED IN THE BUREAU OF CHEMISTRY.

HISTO-CHEMICAL CHANGES IN COLD-STORED CHICKENS.

Gautier [a] states that the muscle fibers of cold-stored flesh remain unaltered except, perhaps, for a slight pulling apart of the individuals. The marked and deep-seated changes, the occurrence of which has been demonstrated in connection with the study in this laboratory, of chickens preserved by cold are, therefore, of interest, and it seems probable that the solving of the problem of the cause, sequence, and ultimate result of such changes would throw much light on the whole question of the various alterations undergone by flesh at low temperatures. While the various dyestuffs which have been used to differentiate the tissues preparatory to microscopic study have not, as yet, served as exact microchemical reagents in the sense of classification of compounds, they have very positively indicated an alteration in the chemical character of certain morphologically distinct elements.

The characteristic green of the normal, fresh muscle fiber, when stained as previously described, is very decidedly altered by long keeping at low temperatures, so much so that finally it is almost entirely replaced by dirty yellow and brown greens, or even by orange tints. The white fibrous connective tissue, staining a brilliant blue in the fresh chicken, in the cold-stored takes on a greenish tint. The material which exudes from the muscles after keeping for even a comparatively short time has, at first, almost the same staining reaction as the fiber itself, but it changes gradually, until it is a dirty brownish or bluish green. The irregular staining of the fiber would also indicate the presence of a progressive chemical alteration. The rupture of the sarcolemma and the extrusion of the muscle substance are not merely mechanical alterations due to freezing and thawing. If such were the case the progressive effects which are so clearly traced in fowls kept for periods of varying lengths of time would be wanting. The differences between the chicken muscle frozen for 48 hours and that of the fresh bird lies chiefly in the size of the spaces between the fibers or bundles of fibers, and deep-seated changes are not seen.

The histological alterations which have been traced are confirmed by the differences noted between stored and fresh chickens in the distribution of the protein nitrogen. It would seem probable that for the chickens stored 2 years and 4 years, respectively, the greater part of the change is due to enzyme action, not only because the tissue shows but few bacteria either living or dead, nor because the histological degeneration proceeds differently from that observed when ordinary temperatures prevail, but also because of the changes

[a] Loc. cit.

in the relative distribution of the protein nitrogen. The fowls in storage 14 months give indications of more marked changes, chemically, than did the others examined, considering the length of the storage period, but such changes may be due to the number of organisms which were found in a living condition in the tissues and which may have been present when the fowl was stored. Because these chickens have an unknown history preceding their entrance into the cold-storage warehouse it is impossible to say whether the numbers found represented an increase or a decrease during the storage period. Such questions can only be answered by the study of chickens of known and of a strictly comparable history.

Neither can this report deal comprehensively with the question of the migration of organisms from the intestine into the edible portions. Judging from the ravages undergone by the walls of the gut during storage it could offer but a slight barrier to active bacteria. From an investigation which is now in progress to determine the resistance of intestinal organisms to cold when in their natural environment it appears that they do remain alive in large numbers. They are, however, of the varieties which develop best at 20° C. instead of body heat, agreeing in this with the behavior of the naturally occurring milk organisms when kept under like circumstances.[a] The fact that the organisms which have been found are not gas producers in dextrose media would argue against possible migration. The location of the bacteria in the tissues, on the other hand, would indicate its possibility since there are fewer in the muscles of the breast than in the inner thigh muscles, which in the chicken lie closely adherent to the body wall.

CHANGES IN FAT.

The decomposition of the fat of the chicken is much more pronounced than is the decomposition of the protein, and in the tracing of the changes which occur in this tissue not only bacteria and enzymes must be taken into account, but light and air must also have due consideration.

It has been held that the splitting of fat into acid and glycerol is the cause of rancidity. More recent studies would indicate that, while such a splitting ordinarily accompanies the condition known as "rancid," it is not the real cause of it, but that rancidity is due to the action of air and light on fats which have been previously split by enzymes acting in the presence of moisture.[b] The part played by bacteria in the decomposition of fats in their natural environment is not a minor one, though the rôle to be assigned to them regarding

[a] Pennington, loc. cit.

[b] Lewkowitsch, Chemical Technology and Analysis of Oils, Fats, and Waxes, 3d ed., 1904, p. 22.

the production of rancidity is still a mooted question. Since bacteria will not propagate in pure, dry fat, but must have present suitable nitrogenous foodstuffs as well as moisture, the conditions for their activity, as pointed out by Lewkowitsch, are the same as for the activity of enzymes, hence it is exceedingly difficult to say where the action of either the one or the other ceases, but it would seem probable that fat splitting can be ascribed to both enzymes and bacteria, whereas the decomposition of the free fatty acids or of the glycerol must be referred to bacteria alone.

The researches of Kastle and Loevenhart on lipase[a] have established the wide distribution of the enzyme in nature and its stability as well as its adaptability to its environment. It was kept dry and moist, and at room temperatures and in cold storage, retaining its activity for months. It hydrolyses most rapidly at 40° C., at which temperature 11.29 per cent of fat splitting was noted; at 10° C. the amount was 3.89 per cent; at 0° C., 2.26 per cent, and at −10° C., 0.70 per cent, a quantity which, given weeks and months in which to augment, might easily reach notable proportions. The enzyme lipase shows a decided preference for fats of higher molecular weight, wherein it differs from the usual acids used to induce hydrolysis. It is also able, under favorable conditions, to reverse its action, and synthesizes ethyl butyrate, for instance, from alcohol and butyric acid. Hanriot[b] states that lipase can form monobutyrin from butyric acid and glycerin and the higher the molecular weight of the acid the more easily is the synthesis accomplished. Such facts indicate a most important and far-reaching physiological rôle for the enzyme, as is discussed at length by Loevenhart,[c] who finds it in considerable quantities wherever fat synthesis is taking place in the living tissue, as in the subcutaneous fat and in the secreting mammary gland. Between the enzyme studied by Loevenhart, in the milk gland, and that occurring in milk, Gillet[d] finds a most important difference, namely, that the latter splits monobutyrin but not ordinary fats. In accord with such a train of thought comes the work of Connstein, Hoyer, and Wartenberg,[e] who found a great variation in the ability of lipase to split fats from different sources. Butter is very resistant, owing, probably, to its high percentage of acids of low molecular weight, since triacetin gave 0.4 per cent, tributyrin 9.5 per cent, and triolein 50.6 per cent of free fatty acid when all were treated under like conditions. It is also noteworthy that the reaction is much more

[a] Concerning Lipase, the Fat Splitting Enzyme, and the Reversibility of its Action, Amer. Chem. J., 1900, *24*: 491.

[b] Sur la reversibilité des actions diastatiques, Comptes rend. soc. biol., 1901, p. 70.

[c] Amer. J. Physiol., 1901–2, *6*: 351.

[d] Existe-t-il une lipase dans le lait?—J. physiol. pathol. general, 1903.

[e] The Enzymic Decomposition of Fat, Abs. J. Soc. Chem. Ind., 1902, *21*: 1541.

rapid in the beginning of the experiment than when there has been an accumulation of the products of activity.

Such wide and varied abilities appertaining to an enzyme as are indicated by the foregoing summary demand that account be taken of its action, wherever natural changes of fat are in progress, and its ability to retain its activity for long periods of time and to work at low temperatures makes its rôle in fat changes in cold storage an important one.

Since enzymes of varied abilities have been traced to a bacterial origin it is quite reasonable to infer that those having some action on fats would be so produced. Schreiber,[a] who has studied earth bacteria, was unable to find ferments, and considers the decomposition of fats to be dependent upon vital functions. It is then affected by all the conditions which affect life. Schreiber states that anaerobes split fats slightly, but their activity ceases at that point and is feeble at best. Rahn[b] denies that anaerobes have any action on fats, but admits that they can decompose glycerin. Both of these authors report work on certain molds—Mucor, Penicillium—and find that they cause both a rapid and a complete decomposition. Among bacteria *B. fluorescens liquefaciens* shows a fat-decomposing power closely following that shown by molds. Rahn[c] has determined this activity as expressed by the iodin number, which was considerably increased for palm fat, stearin, and butter fat. Laxa[d] has recorded analyses covering the values usually determined in fat examinations using butter fat with molds, yeasts, and fat splitting bacteria proper. The latter, with the exception of *B. fluorescens liquefaciens*, commonly cause a decrease in the iodin number; the other fungi noted usually increased it. The Reichert-Meissl number was but little changed. The acid value increased for both molds and bacteria. The saponification number decreased with both. The index of refraction in many instances fell when the iodin number rose, and when the latter decreased the refractive index remained stationary. The Hehner number for molds showed a rise.

It will be observed that there is a distinct difference between the action of molds and of bacteria on fats. This is again expressed in the work of Girard, who believes that Aspergillus and Penicillium act by way of an enzyme and Laxa[e] has demonstrated by experiment that such is the case. With substances as complex as are fats in their natural environment and with forces as delicate as fungi

[a] Fettzersetzung durch Microorganismen, Arch. Hyg., 1902, *41*: 328.

[b] Die Zersetzung der Fette, Centrbl. Bakt., 2 Abt., *15*: 53.

[c] Loc. cit., *15*: 422.

[d] Ueber die Spaltung des Butterfettes durch Microorganismen, Arch. Hyg., 1902, *41*: 118.

[e] Loc. cit.

and enzymes, and with such universal factors as air and light to be considered, it is not to be wondered at that the whole question of fat decomposition in nature is still greatly in need of elucidation. The literature on the subject, as indicated in the foregoing discussion, is chiefly concerned with plant oils and butter fat and with fats freed from the tissues normally occurring with them. Almost invariably, also, the studies made have been conducted at body or at room temperature. The discussion of the changes in the fat of cold-stored chickens as indicated by the variation between their values and those of the fresh birds, which values are given in another section of this report, becomes therefore a difficult task. Light, as a cause of decomposition, need scarcely be considered; air, at least for the superficial fats, is probably a factor and would tend to increase the acidity. So great are the differences between the cold-stored and fresh chickens when the acid value, saponification number, and ester number are considered that probably not only air but enzymes as well, and perhaps bacteria too, have all played a part in the alteration. The lowering of the iodin number rather argues for a certain amount of bacterial action, as does also the decrease of the saponification number, since the available contributions on the subject agree in assigning such results to bacteria. The change in the Hehner number due to bacterial action is not discussed to any extent in the literature. Laxa,[a] in his study of butter, makes two determinations, one for *Oidium lactis* and the other for *B. fluorescens liquefaciens*, finding an increase in both cases. The Hehner number for fresh chicken fat is lower than is usually found for animal fats, and after cold storage a further decrease is observed. It is difficult to explain the cause of a rise in the Hehner number as a result of fungoid action, since that is essentially katabolic, and not at all of the character to produce acids of a higher type than those normally present, whereas the decomposition of insoluble acids with the production of soluble forms is perfectly logical and quite in line with the observations of Rahn and Krueger.

SUMMARY OF RESULTS.

(1) Macroscopic observations of fresh and cold-stored chickens show that certain plainly visible differences exist between the two classes, which differences are progressive, depending on the length of the storage period.

(2) Chemical analyses of fresh and cold-stored chickens, wherein were determined the total nitrogen for both dark and light meat and its distribution between the classes of compounds commonly accepted as the result of protein hydrolysis, the various values from which a knowledge of the composition of fat may be obtained and such

[a] Loc. cit.

minor constituents as water, ash, etc., have served to show that for the protein distribution there is a slight variation in the cold-stored product from the fresh, and for the fat values a wide variation.

(3) A histological examination of the muscle of both fresh and cold-stored chickens shows a marked and a progressive change in the structure of the fibers which is deep-seated and after long periods renders the tissue almost unrecognizable. Selective microchemical differentiation of the tissues confirms the chemical change found by gross analytical methods.

(4) A bacteriological examination of fresh and cold-stored chickens reveals the presence of appreciable numbers of organisms, calculated on the gram basis, in the edible portions of those preserved by cold, though the numbers were not large. In fresh fowls the same technique gave no bacterial growth.

GENERAL REVIEW OF THE INVESTIGATION.

In reviewing the details of this preliminary work it must be remembered that some of the inferences drawn may be modified by future investigations, although the fact that certain changes take place in the food materials examined after definite periods of storage is well established both by the organoleptic and by the chemico-bacteriological tests and is further confirmed, in the case of the chickens examined, by the histological studies.

The principal lines of investigation reported, namely, on eggs, quail, and chickens, conducted at Washington under known conditions of storage, and the more complete study of chickens stored under market conditions at Philadelphia, are summarized in the following pages, brief reference also being made to the conclusions drawn from the milk investigation previously reported by Pennington.

ORGANOLEPTIC TESTS ON CHICKENS AND QUAIL.

The general results of the organoleptic tests of the stored fowls and birds leads to the conclusion, first, that even after three months, before cooking, it is not difficult to distinguish, by the appearance, color, and odor, a freshly killed bird from the one that has been in cold storage. The shriveled condition of the eye and the skin and the generally dilapidated appearance of the bird are very significant and distinct. After cooking, however, within a period of three months, there is much disagreement on the part of the jurors respecting the proper classification thereof. This is especially true as to distinguishing between the drawn and the undrawn birds, where the variations in judgment are of such a character as to lead to the belief that it is impossible within that time, from the taste, odor, and smell of the cooked bird, to determine which one had been drawn and which was undrawn at the time of storage. The possibilities of determining

which is the fresh bird are very much greater, even after a lapse of three months, although occasional mistakes may be made in this respect also. At later dates, such as at the end of six months, nine months, or twelve months, the difference between the birds becomes more and more pronounced, so that it may with certainty be said that even after cooking one would rarely confound a fresh bird with one which had been in storage six months. At that date the flavor and the general character of the meat have so deteriorated that it is not difficult to distinguish between the fresh and the stored fowl. Even at this date, however, there is some difficulty in distinguishing between the drawn and the undrawn bird. At the end of a year or more it would be quite impossible to make a mistake in most cases between the fresh and the stored birds.

Summing up the organoleptic properties, it may be said that for a short time, possibly six weeks or even longer, there is no perceptible change produced in a chicken by having it frozen. There certainly does not seem to be any evidence that it is better, and there is no convincing evidence that it is any worse. After three months, however, the fresh chicken is easily distinguished by its properties, as a rule, from the cold storage chicken, even after cooking, and to an absolute certainty before cooking. This distinction between the fresh and the stored bird becomes more and more marked as the time of storage is increased. In so far as the drawn and undrawn chickens are concerned there is much less certainty of being able to distinguish between them. However, 70 per cent of the jurors were able to pick out the undrawn bird by its stronger odor and taste after a storage period of from six to fifteen months, but at the test representing 18.5 months' storage the two birds were about equally dry and tasteless.

The general conclusion is, therefore, that in the case of frozen birds there is no indication of any improvement in quality, that is, in taste, odor, or flavor, during cold storage. There is a deterioration which is noticeable, even at the end of three months, and becomes more marked as the time of storage grows longer. Hence, without any reference whatever to the question of wholesomeness, cold storage prolonged for six months or more appears to be distinctly detrimental as far as taste, flavor, and palatability are concerned.

BACTERIOLOGICAL AND CHEMICAL INVESTIGATIONS.

MILK.

As a part of this investigation certain studies have been made on the changes taking place in milk when kept at low temperatures. The detailed report of this study has already been published,[a] hence

[a] J. Biol. Chem., 1908, 4: 353, Bacterial Growth and Chemical Changes in Milk Kept at Low Temperatures, M. E. Pennington.

there will be included here only the conclusions reached, which are as follows:

SUMMARY OF RESULTS.

Bacteria in milk increase in numbers when the temperature is maintained at or a little below 0° C. This temperature is below that ordinarily assigned as the limit of bacterial multiplication.

Milk has been kept for periods ranging from a few days to almost two years at a temperature of 29° to 31° F., and also at 32° F. It has been kept in a packages of ten quarts and one quart. It has been the cleanest milk obtainable, by the most carefully enforced refinements of modern dairying; and it has also been market milk produced in the ordinary dirty stable and subjected in transit to the usual careless handling.

Bacterial growth at the end of a week, even in the cleanest milk, which contained as low as 300 organisms to the cubic centimeter, was pronounced. There was a steady increase in the number of organisms for five or six weeks, and at their maximum they numbered hundreds of millions. Occasionally they passed the billion mark per cubic centimeter.

Continued exposure to a temperature of 29° to 31° F. causes, after a lapse of from seven to twenty-one days, the formation of small ice crystals which gradually increase until the milk is filled with them and there may be an adherent layer on the walls of the vessels. The milk does not freeze solidly. In spite of the fact that the milk was a semisolid mass of ice crystals, the enormous increase in bacteria which this study shows took place. Though the bacterial content was numerically in the hundreds of millions per cubic centimeter there was neither odor nor taste to indicate that such was the case. Neither did the milk curd even on heating, and it was not until the bacterial content began to fall, and organisms of putrefaction were under way, that the use of the milk for household purposes would, to the ordinary observer, become contra-indicated.

A classification on a chemical basis of the organisms occurring at these low temperatures shows that there were constantly present bacteria which formed acid and bacteria which acted upon proteid. There were also neutral organisms, which formed neither acid nor alkali and did not act upon gelatin. The acid-forming organisms were generally in relatively smaller numbers than are found when milk is kept at higher temperatures, and the liquefying organisms were more numerous. Certain species, such as *B. formosus*, *B. solitarius*, and *B. raveneli*, were especially resistant to cold and frequently were the predominating species, or almost in pure culture at the close of the experiment.

A very marked difference in both the number and kind of organisms which developed on the plates was noticed, depending upon the temperature at which the plate was incubated. In certain experiments the maximum number grew at 37° C. In others the temperature at which the milk was stored served best for colony formation. The relative number of organisms growing at 37° C., 20° C., and 0° C., or a little below, varied greatly also with the length of time that the milk had been kept in storage, the organisms developing at body temperature being ordinarily greatly in excess at the beginning of the experiment and diminishing until near its close, when a sharp rise was apt to take place.

The determination of the acidity showed that after a few weeks a much higher acid content was reached than is ordinarily required for the spontaneous separation of curd, which, however, seldom happened. Milk having this high acidity, when placed in an ordinary ice chest, increased in acid content but did not curd for days after exposure to the higher temperature.

The chemical study of the proteid of milk in cold storage showed that the casein was rapidly digested until finally more than 50 per cent of it was changed to soluble compounds. Caseoses, amido acids, and probably peptones, increase apparently at

the expense of the digested casein. The rapidity with which this digestion takes place varies in different samples, but at the expiration of two weeks it is pronounced.

What the effect of the low temperatures is on the carbohydrate constituents of the milk remains for further study. That an interesting decomposition, and one which varies from that occurring at higher temperatures, takes place is indicated by the very high acid content of the milk noted throughout this investigation.

Similar studies, conducted on samples of very fresh milk kept at the temperature of the laboratory (about 18° to 22° C.), show a very decided difference chemically from the decomposition of milk in cold storage. Bacterial growth at room temperature is, of course, rapid and profuse. The acid-forming organisms are, as has been found by other observers, in high proportion and the liquefying organisms are relatively lower. The chemical change observed is, by comparison with that occurring in cold storage, almost nothing. At the curding point only about 1 per cent of the casein has been changed to soluble products, and spontaneous curding is observed, ordinarily, when the acid content falls between 23 and 28 cc of tenth-normal sodium hydrate per 100 cc.

CHICKENS.

The results obtained show unmistakable evidences of bacterial activity during the period of storage. While naturally the frozen condition of the flesh prevents to a great extent bacterial migration, it does not prevent absolutely bacterial growth. It is perfectly certain from these experiments, as well as from others, to which reference has been made, that a degree of cold, equivalent to that sustained in ordinary cold storage, does not by any means destroy bacteria. The effect is simply to produce a state which may be described somewhat broadly as suspended animation. It is also certain in some instances that the number of bacteria may be diminished during cold storage, showing that while cold does not kill all species of bacteria it may hold certain forms in suspended animation so long that they are finally killed.

Attention should be called also to the fact that as this line of work was entirely new, a great deal of the time has been devoted to determining the best methods for the investigation and establishing the general principles on which it should be based. It is evident, therefore, that the deductions which are to be drawn here are only to be considered of a tentative nature and should not be regarded as the final word on the subject. Too few specimens have been examined to make the data sufficiently extensive to be relied upon as a final basis of conclusions. The experiments have undoubtedly been carried on under conditions which are more favorable than those existing in the markets in general.

The fowls on which the work conducted at Washington was done were specially selected, killed with the greatest care, packed with the utmost precautions, and in every way subjected to the cleanest conditions. On the contrary the fowls which are ordinarily placed in cold storage are not selected fowls, they have not been killed and prepared with special care, nor are any special means taken to pre-

vent possible infection. It is evident, therefore, that the data which have been obtained from the bacteriological study of the market cold-stored chickens, as conducted at Philadelphia, must be accorded full value, and the acknowledgment made that with ordinary cold storage of fowls under market conditions the bacterial flora is likely to be very greatly increased.

In the examination of the market cold-storage chickens there was observed a very definite correlation between those fowls in which the quantitative bacteriological findings indicated a relatively large number of organisms and the gross chemical analysis of the bird so far as the degradation of proteids was concerned. Here also the bacteriological and chemical findings were abundantly confirmed by histological sections of the tissues, which showed an invasion of the individual fibers by masses of organisms which had undoubtedly altered the chemical composition of the muscle fibers themselves to such an extent that finally their chemical integrity was absolutely lost. For the detailed summary of the various phases of the investigation of market cold-stored chickens reference should be made to the close of the special report on this subject, page 94.

Among other considerations the proper removal of the viscera from the cavity of the drawn fowls is a matter of great consequence in case they are to be kept in this condition. In point of fact, however, a very small percentage of the fowls which are placed in cold storage are drawn, so that the precautions for the removal of the intestines without danger of infection are of no very great consequence. In drawn fowls, however, such as were half of those stored for the Washington experiments, the utmost care was exercised to prevent infection from the intestinal contents during drawing. This is a danger which the advocates of drawing fowls have, as a rule, overlooked. It is evident that without proper precaution very grave danger of intestinal infection may attend the drawing of fowls.

Attention must also be called to the fact, which seems to be well established by the investigations, that food products which have been kept a long while in cold storage, even if they are sterile, are much more sensitive to infection and the rapid development of deleterious substances due thereto than when in the fresh state. It is commonly conceded to be a general practice in withdrawing cold-storage chickens or other birds from the warehouse to place them in very cold water until the flesh is thawed. This has a double purpose of preventing too rapid thawing, which would be somewhat destructive to the tissues, and at the same time of permitting the absorption of the water which has been lost during the time of storage. During this period it is evident that the carcasses must be subjected to infection. Subsequent to this preliminary treatment the chickens are placed upon the shelves for sale and may remain there a long time, depending

upon the state of the market, before finally reaching the consumers' hands. After this they remain for some hours in the hands of the consumer before cooking. It is easy to conceive of a period of three or four days covering all of these various operations. All this time these materials, so sensitive to infection, are exposed in various ways to danger.

In the consideration of the bacterial studies it must be borne in mind that it is not impossible to find bacteria in the tissues of the fresh fowls. While it is commonly accepted that the general circulation of animals in a state of perfect health is free from bacteria, it is quite certain that in abnormal conditions bacteria may exist in the organs of the body and even in the blood, hence the detection of bacteria in the tissues of a cold-storage fowl, or any of its organs, may not be conclusive proof that these bacteria were developed during cold storage, since they may have been there in the original state in which they were packed.

The findings of the macroscopic investigations emphasize and confirm the organoleptic tests previously discussed in that there was observed a decided fecal and irritating odor, suggesting rancid fat, in the case of the undrawn fowls long in storage, existing coincident with the taste which enabled the jury to distinguish between the two methods of dressing. On the other hand, in the drawn fowls there was a tendency to a rancidity of the fatty portions of the abdominal region and a development of an odor somewhat similar to that produced by a butyric fermentation. In general, however, in so far as the bacterial investigations have proceeded, there is little choice between the drawn and the undrawn fowl. It is possible that on longer keeping there will be developed, in from one to three years, important differences between the drawn and undrawn fowls which will enable them to be distinguished, both by their bacterial flora and by their organoleptic and chemical properties.

HISTOLOGICAL STUDIES.

EGGS.

As is well known, the flavor of eggs begins to deteriorate very soon, even when they are kept cold. In the storage of eggs, moreover, the temperature must be kept above the freezing point—that is, the freezing point of an egg. The egg, on account of its constitution, does not congeal at the temperature at which water becomes ice. It is possible to cool eggs considerably below 32° F., at least for a short time, without freezing them. Nevertheless, it is not safe, as a rule, to go very much, if any, below the freezing point of water. Kept in this way, eggs undergo certain changes, not only of a histological nature, but of a chemical character, which are now under investigation. Certain constituents of the egg have a tendency on storage of this

kind to become crystalline. In the course of a few months eggs which are kept in cold storage are found to develop small rosette crystals in the yolks. These, it has been found, occur most frequently in the outer portion of the yolk. The size of the crystals is found to be from 18 to 109μ in diameter, and the form is shown in Plate III. The exact character and composition of these crystals has not yet been ascertained, as they constitute a relatively small percentage of the entire mass, and hence their isolation in sufficient quantities for analysis is attended with extreme difficulty. Many attempts, as has been seen from the detailed statement in the preceding section of this report, have been made to isolate and prepare these crystals for identification. It is perhaps possible that they belong to the class of substituted fatty bodies, but no definite statement can be made except that it seems that they are not tyrosin. The observations of these bodies seems to be entirely new, as no account has been found of them in other publications. It is probable, therefore, that their existence may be regarded as one of the means of distinguishing eggs which have been a considerable time in cold storage from fresh eggs.

CHICKENS.

Quite as important, and perhaps even more instructive than the studies relating to bacterial growth at low temperatures, are the studies of the changes which take place in the tissues of the material, and which can be recognized under the microscope. Changes in tissue are usually due either to fermentation or to enzymic action, if the two terms are to be used in any different sense. It is generally conceded by investigators at the present time that the actual changes in the structure of the tissues are not so much due to direct action of the bacteria as to the enzymic ferments which bacteria secrete or produce or which exist in the normal cells of the tissue itself. Hence the action of enzymes may be regarded as the last step of fermentation.

The studies in histological changes were undertaken on market chickens which had been stored for varying lengths of time at about 13° F. One difficulty in connection with a general investigation of this kind is that if chickens are selected at random, or purchased in the open market after having been dressed, a good deal of uncertainty must exist concerning their previous history and the conditions to which they have been subjected. Commercially it is well known that the interval which exists between the period of slaughter and the period of storage is of varying length. The temperature and other conditions of environment to which the slaughtered chickens are subjected in this interval are far from uniform. The method of transportation may have much to do with the character and extent of the changes before cold storage in a warehouse begins.

Chickens may be, for instance, prepared for shipment at distant points and sent in refrigerator cars either frozen or at a low temperature on a voyage requiring a long period of time before they finally reach the depot in which they are preserved until a demand for consumption arises. It was necessary, therefore, in all studies of market chickens to begin the work with fowls whose history was known so that they could be followed, not only from the time of slaughter, but even before that time. The different breeds to which the chickens belong, the methods of hatching and raising, the character of the feed which they eat—all may have important bearings upon histological and chemical, as well as upon bacteriological, data. Hence the desirability of the practice which was finally adopted, not only of studying chickens in a commercial way, but also of having those of known breed and history.

Manifestly it was impracticable to study microscopically all of the edible parts of the bird at this time, hence it was decided to select for comparative work the fibers of the large muscle of the breast, the *Pectoralis major*, which is usually considered the choicest food portion, and which is apparently the part which keeps longest and best. In order that the character of the changes may be more readily appreciated, they have been reproduced by microphotographs and by colored plates, so that the reader who is not skilled in microscopic work nor in histological investigations may be able to see at a glance the character of the changes which have taken place. (See Pls. VI to XI.) The sinuous outline of the fresh fibers soon disappears in cold storage, and the fibers are not by any means so flexible as they were in the fresh state. The most obvious change, however, which takes place during this interval is manifested as a structureless, granular substance which lies between the individual fibers and between their aggregations which are known as bundles. The origin of the substance is in the fiber itself, and in the earlier periods of storage its composition, according to reactions obtained with various selective dyestuffs which afford excellent microchemical reagents, is not very different from that of the normal fiber. As the storage period is lengthened, however, most marked differences between the normal and cold-stored tissues, as exhibited in the staining reactions, make their appearance, and such changes, whether referable directly to bacterial or enzymic action, are essentially chemical, and deal with the fundamental principles composing flesh foods. The results obtained from this microscopic study of the histology of market cold-stored fowls would seem to point the way to a series of investigations, which has already been begun and from which it is believed will accrue a knowledge of the obscure chemical changes undergone by protein, to elucidate which the present gross chemical methods are entirely inadequate.

Such changes as were observed in the flesh of these cold-stored fowls make it especially desirable that there be carried out certain pharmacological experiments, using preparations of cold-stored tissues. It is held by some of our most eminent medical authorities that many severe intestinal disturbances are traceable to cold-storage animal products, particularly when the viscera are not removed before storing. Hemmeter[a] says in this connection: "I have personally observed numerous cases of sudden and severe auto-intoxication from the gastro-intestinal tract which I could interpret in no other way but that they were due to the ingestion of cold-storage food."

In view of the indisputable fact that changes do take place in flesh at low temperatures, and considering the authority lent to the above statement by the prominence of its author, it would seem most necessary that further experiments based on strictly scientific principles be prosecuted looking toward either its refutation or explanation in order that the evil may be remedied, if it exists. The intestines, which are left *in situ* in storage birds, show a very marked degeneration. Their muscular walls grow thinner in cold storage until they are the merest remnants, which threaten to disappear altogether and which even very careful handling may easily rupture. This degeneration is noticeably active in the muscular rather than in the cellular tissues of the intestines. This is important when it is considered that the bacterial flora of the intestinal contents will, of course, contain any pathogenic germs which usually accompany the colon bacillus. Hence the perforation of the walls of the intestines, which apparently takes place by continued digestive processes even in cold storage, would open the way for a rapid migration of such bacteria on thawing and previous to cooking. Thus it is quite possible that dangerous bacterial organisms might be translated to the edible portions of the fowl through the perforations of the intestines in the period between thawing and cooking. This degeneration of the walls of the intestines must, therefore, be regarded as highly significant.

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[a] Memorial number of the Maryland Medical Journal, June, 1905, *48*: No. 6.

APPENDIX.

TREND OF LEGISLATION REGULATING THE COLD STORAGE OF FOODS, AND OPINIONS EVOKED THEREBY, ESPECIALLY WITH REFERENCE TO DRAWN AND UNDRAWN POULTRY, ETC.

GENERAL DISCUSSION AROUSED BY PROPOSED CHICAGO ORDINANCE.

In 1906 an ordinance was introduced in the Chicago council to exclude from the city trade all undrawn poultry or animals as food products.

In the course of the discussion which this bill evoked Runnels and Burry, attorneys for the cold-storage warehouse interests, issued a pamphlet dealing not only with the question of drawn and undrawn poultry, but with cold-storage conditions in general, and from which the following extracts are taken.

In regard to the quantity of material stored they state:

> About twenty million pounds of poultry are cold stored in Chicago each year, and there are stored proportionate amounts of butter, cheese, meats, game, eggs, and other food products.

In a letter from a large western firm the statement is made:

> We bring to the city of Chicago for storage annually 6,000,000 pounds of poultry, 300 carloads of eggs, and 100 carloads of butter, * * * 5 per cent is sold for Chicago consumption. The other 95 per cent is sold for consumption in cities outside of the State of Illinois or abroad.

During the month of January of the year 1906 this firm shipped to Liverpool and London 1,000,000 pounds of poultry. The business of this firm during the year then just past (1905) had exceeded $1,500,000.

In dealing with the question of the length of time the various cold-stored products should be or may be carried, the pamphlet cited makes the following statements:

> Eggs and apples, which are perhaps stored more extensively than any other commodities, can not by any system of cooling or refrigeration be kept a year in cold storage, and they are usually taken from storage in a much shorter time than that. Probably few apples are kept over six months in cold storage. * * * Eggs are put in storage during the months of April, May, June, July, and a few in August. April and May eggs keep best. In selling them the owner sells the latest eggs first, so that July and August eggs are never in storage more than from two to four months, while April and May eggs are closed out usually during December and January. Some eggs remain, therefore, in storage more than six months, but the rule is that they are to be disposed of by the first of February.

Poultry is sent to market, dressed and stored, during the months of October, November, and December. The amount so stored, killed when poultry is in its best condition, is intended to supply the market with the best goods obtainable until the new stock comes in during the next receiving season. Necessarily, therefore, part of this stock is carried for more than six months.

In the letters forming a part of this pamphlet the statements on this phase of the question are quoted as follows:

When poultry is to be frozen, the best stock in the market is generally selected; and if the same is killed and cooled properly, it can safely remain in cold storage for fully two years, if necessary, without becoming tainted and injurious to health. However, it is seldom, or, I might say never, kept longer than eight months. * * *

Poultry that is dressed and cooled off properly before going into cold storage will come out in good wholesome condition any time, within at least one year, and it would take a very good expert to tell the difference between good fresh frozen poultry and fresh killed at time of eating.

Another dealer states:

Poultry with entrails in it can be kept in our modern freezers, where they shall be frozen and kept at a very low temperature, and be held in storage one, two, or three years, or even longer, without any detriment to the poultry itself, and on such poultry there is no possible chance of there being any cause for fear of ptomaine poisoning.

Another warehouseman in a letter states:

Poultry placed in a freezer under proper conditions and kept at the right temperature will keep an indefinite length of time and in healthful condition.

Taking up the question of drawn and undrawn poultry, the attorneys for the warehousemen came to the conclusion that—

The experience of men well versed in the trade as to the desirability of drawing poultry before storing, it is best shown by the letters printed herewith. From them it will be seen that drawing poultry before putting it in the freezer is impracticable. It will not keep as well drawn as undrawn.

From the letters to which reference is made we find the following statements:

We have experimented with poultry in every form. We have found that it is utterly impracticable to carry drawn poultry in the freezer. It is very similar to any other product in that so long as it remains an air-tight package it can be carried much better than when it is open to the atmosphere, which dries it, discolors, and generally injures the inside texture of the bird when exposed. Further, of the markets which buy the frozen poultry from us there is not one that can or will accept it drawn; and although there is on the statute books in the State of Massachusetts a law demanding that all poultry marketed dressed shall be drawn, the same is a dead letter, and all our shipments to Boston and other Massachusetts cities are undrawn.

Again, another dealer states:

We are handling every year a small portion of storage poultry that is drawn, heads and feet off, and we usually have to sell this class of goods at from 1 to 3 cents below the price of the poultry that is undrawn with heads and feet on. I feel satisfied that if we had to draw our poultry and depend on our customers to sell the same to, we would have to quit the poultry business, as we certainly could not compete with other cities that are selling undrawn poultry. I might say that I do not know of any market in the United States where we would be able to sell drawn poultry, even at a discount of from 1 to 3 cents per pound less than the undrawn poultry.

In another letter occurs the following passage:

A chicken when dead and undrawn is hermetically sealed, and you do not find the decomposition the greatest in the inside of the bird, but you do find it between the legs and the body and under the wings.

In still another letter the warehouseman states:

The result of having to draw poultry in the country for shipment into Chicago would cause the greater percentage of the poultry to arrive at its destination in a more or less putrified condition, and such poultry put into storage, no matter under what conditions, even with the use of modern refrigeration, the inside of the poultry (the fact of its having been wet) will become moldy, either showing mold with black spots or with a woolly fungous growth.

That opinion is not unanimous in regard to the advisability of retaining the entrails in dead animals until the time of cooking shall arrive is indicated by a number of public statements from various sources, certain of which are appended.

VARIOUS OPINIONS ON DRAWN AND UNDRAWN POULTRY.

Franklin G. Fay, of Sacramento, writing on the subject in the California State Journal of Medicine, 1904, Volume IV, page 66, states that—

Resolutions condemning the use for human consumption of fish and poultry from which the viscera were not removed at the time of slaughter were passed by the Sacramento city board of health, and that such legislation, being before the Sacramento board of trustees, embodied in the form of an ordinance, was meeting with strenuous opposition on the part of the dealers, led by the large packers and cold-storage men.

Doctor Fay says:

If their immense interest in this matter is really in behalf of the public, we should have no difficulty in making a satisfactory adjustment, but as yet we have found no consumer outside of the trade who is opposed to legislation. * * * The reports of the Canadian commissioners of agriculture show that the requirements of the English market demand that the intestines be removed.

Among the very prominent opposers of the custom of marketing undrawn poultry is Doctor Cavana, of Oneida, N. Y., who makes the following statement in a paper entitled "Dangers in Undrawn Poultry and Game," presented at the annual convention of the American Association of Railway Surgeons at Chicago, Ill., October 17, 1906:

Bacterial cultures made from the breasts and legs from 100 undrawn fowls proved the presence and thorough permeation of the tissues in each specimen, with the various groups of intestinal germs, some tests showing no less than eleven distinct groups in one poultry specimen. Among the varieties of pathogenic bacteria identified was the *Bacillus coli communis*, the *Staphylococcus pyogenes aureus*, the *Bacillus proteus vulgaris*, and the *Streptococcus pyogenes*. Previous tests of intestinal matter taken from the entrails of recently slaughtered fowls revealed the presence of these latter germs in great abundance in the intestinal canal of every fowl examined, and their discovery in the remote tissues of the undrawn cold-storage specimens proves un-

mistakably that their source was from the alimentary canal, and their permeation of the tissues the direct result of the retention of the intestinal tract and its contents in the sealed abdominal cavities of the poultry carcasses. Growers of poultry will support us in the assertion that the ducks and hens of the barnyard are the scavengers of the farm. They are constantly picking over the soil, the farm garbage heaps, and other bacterial hotbeds, and no accretion or mass of decaying matter ever becomes too repulsive for poultry food, especially that of hens. In our bacteriological studies all of the cultures were made from the eatable tissues of the various specimens and with the most thorough aseptic precautions. The *Bacillus coli communis* was found in 100 per cent, or every specimen tested; the *Bacillus proteus vulgaris* in 6 per cent; the *Staphylococcus pyogenes aureus* in 20 per cent; and the *Streptococcus pyogenes* in 65 per cent of the 100 examinations.

Probable infection before storage.—The cold storage plant owners of New York City inform us that their poultry stocks are collected from all parts of the country, even as far distant as the States of Texas, Louisiana, and Florida. After slaughter the feathers are removed and the carcasses packed in barrels without further dressing. The head, feet, and legs, as well as the craw of partially digested food, and the decomposing livers, lungs, and intestines with their filthy contents, all combine to make valuable weight, and are therefore left in the sealed cavities of the fowls, forming conditions which force the general infection of the tissues by the flagellated, or rapidly swimming intestinal bacteria, which double their quantity and numbers every forty minutes, a single bacillus being capable of developing over forty-two billion germs in twenty-four hours. Their shipments are made by rail and steamship, and cover transit periods of several days before reaching the cold atmospheres of the storage warehouses.

To determine the activity of these germs and the period required for their permeation of the tissues in the slaughtered undrawn fowl, we caused to be made a series of experiments, the results of which justify the belief that a great percentage of the infected poultry and game stock in storage became so infected before reaching the low temperature of the storage warehouses.

As a direct reply to the above-mentioned work of Doctor Cavana, Dr. Henry A. Higley, at the solicitation of the New York Poultry Dealers' Association, made an investigation and presented the same at a hearing before the legislative committee at Albany, on behalf of the opposition to a bill introduced by Doctor Cavana providing that all poultry must be drawn within eighteen hours after killing. Among Doctor Higley's conclusions are the following:

1. No matter how many of the bacteria which are concerned in this discussion there may be in the intestinal and thoracic cavities of dead undrawn poultry and game, they can not invade the edible portions so long as the temperature of such poultry and game is kept at 5° C. (41° F.) or below, because these bacteria do not grow at such a temperature or below.

2. Dead, undrawn poultry and game kept at a temperature (above 41° F.) which would allow the invasion of its edible portions with these bacteria mentioned from the intestinal tract would be subject to putrefaction changes because such a temperature would be much more favorable for the growth of the bacteria which produce putrefaction, since they can grow at as low as 0° C. (32° F.).

3. Even if the bacteria invasion claimed by the supporters of this measure does take place, such bacteria can produce no poisonous substances because they are placed under unfavorable conditions of growth so long as the temperature of the fowls is kept anywhere near 5° C. (41° F.).

4. The longer dead poultry and game is kept frozen the less bacteria will it contain, because freezing temperatures gradually destroy bacteria.

And as a general conclusion to the whole matter he makes this statement:

All bacteriological evidence conclusively proves that the edible portions of healthy, dead, undrawn poultry and game do not contain any bacteria, toxines, or ptomaines that are harmful when eaten by man, so long as such poultry is kept free from putrefaction. That poultry that goes into cold storage in good bacterial condition comes out in exactly the same condition that it went in, so long as the temperature of the poultry is kept low enough to prevent the growth of putrefactive bacteria and, finally, that the longer poultry remains frozen the less bacteria does it contain.

SUGGESTIVE LEGISLATIVE ACTION.

MASSACHUSETTS ENACTMENTS.

That the question of the advisability of storing fowls in an undrawn condition is one of widespread interest is evidenced by the localities which have made this matter a subject of investigation and have finally presented it to their legislative bodies. For instance, in Massachusetts a bill was introduced relative to the sale and storage of poultry, in which it has recommended that—

Whoever, himself or by his servant or agent, or as the servant or agent of any other person, firm, or corporation, sells, exposes for sale, exchanges or delivers, or has in his custody or possession with intent to sell, exchange, or deliver, any dead poultry from which the head, crop, if it contain food, and the entrails have not been removed, shall be punished by a fine of not less than five nor more than fifty dollars for each offense; but the provisions of this section shall not apply between the first day of April and the thirty-first day of October in the case of what is known commercially as "Iced poultry," that is to say, poultry shipped in ice.

Whoever, himself, or by his servant or agent, or as the servant or agent of any other person, firm, or corporation, places, or causes to be placed, or holds in cold storage for purposes of sale, any poultry from which the head, crop, if it contain food, and the entrails have not been removed, shall be punished by a fine of not less than twenty-five dollars for each day said poultry is held as aforesaid. For the purposes of this and the preceding section, the word "poultry" shall be deemed to mean ducks, geese, turkeys, fowls, and chickens.

The result of the introduction of this bill was the passage of a resolution providing for a comparative investigation of drawn and undrawn poultry when shipped or stored. Dr. Charles Harrington, secretary of the board of health of Massachusetts, undertook to carry out the investigation which was recommended, and has submitted a report in which he draws the following conclusions:

1. During cold storage at from 15 degrees below to 5 degrees above 0° Fahrenheit no chemical changes occur. This is shown by the absence in both the drawn and undrawn birds of ptomaines and decomposition products in general, and by negative reaction on the part of animals inoculated with extracts obtained from both kinds of material.

2. When removed from cold storage and exposed to ordinary temperatures, the condition of exposure being the same, the undrawn birds show better keeping qualities.

3. Freezing renders the muscular tissues more susceptible to bacterial invasion after they are thawed out.

4. The usual method of drawing poultry leads to heavy bacterial infection, which promotes more rapid decomposition than occurs in undrawn birds.

5. By ligature of the gullet below the crop, poultry can be completely drawn without any spilling of the intestinal contents, with consequent bacterial invasion of the abdominal cavity; and poultry so drawn would undoubtedly withstand decomposition and deterioration much longer than that which is undrawn.

6. The practice of depositing poultry in cold storage when in the beginning or advanced stages of decomposition, in order to save it, is dangerous to the health of the consumer, since when it is again withdrawn for sale its condition is unaltered.

7. Proper and adequate inspection of poultry as it enters cold storage is desirable, and storage of material already in process of decomposition should be prohibited.

8. The practice of placing cold-storage poultry in cold water for a number of hours for the purpose of thawing causes heavy bacterial infection and consequent more rapid decomposition than occurs when thawing is allowed to proceed slowly at room temperature. Such treatment causes also a material increase in weight, by reason of absorption by the tissues of water, to the detriment of the purse of the purchaser, and hence is fraudulent.

SACRAMENTO, CAL., ORDINANCE.

The city of Sacramento has an ordinance relating to and regulating the sale of undrawn, slaughtered fish, game, and any animal to be used for food purposes within the limits of the city of Sacramento, and has prescribed a punishment for the violation of the provisions thereof. This ordinance forbids any person, firm, or corporation to sell, offer, or expose for sale any slaughtered animal used for food purposes, refrigerated or otherwise, which has not been properly drawn and prepared by removing the viscera at the time of slaughter, and any person, firm, or corporation failing to follow the provisions of this ordinance is deemed guilty of a misdemeanor, and punished by a fine not exceeding $100 or by imprisonment in the city prison not exceeding fifty days, or by both such fine and imprisonment. This ordinance went into effect on and after the date of February 1, 1905.

ENACTMENTS IN ILLINOIS.

The Illinois State board of health has issued a pamphlet to the municipalities of the State of Illinois strongly recommending that there be enacted and enforced ordinances prohibiting the sale of fish, game, or any animal used for food which has not been properly drawn and cleaned at the time of slaughter. A paragraph in this pamphlet reads as follows:

It is known to all physicians and physiologists that there are generated in the body of any animal poisons of the highest degree of toxicity. The intestines and other digestive organs contain at all times materials which have undergone putrefactive changes. If this material be permitted to remain in the body after death, the poisons generated may infiltrate the entire flesh, making it dangerous to the person who eats it. The body in which the viscera are permitted to remain undergoes decomposition much more rapidly than when such viscera have been removed. Decomposition is further hastened by leaving the blood in the vessels of the body.

Dr. E. N. Eckard, commissioner of health of Peoria, Ill., caused to be instituted an examination of drawn and undrawn chickens, such as were found offered for sale in the markets of Peoria, coming from cold storage plants. On the basis of the reports submitted to him by the bacteriologists in whose hands the practical investigation was placed, an ordinance was passed by the city council of Peoria, which reads as follows:

SECTION 1. That it shall be unlawful for any person, firm, or corporation within the limits of the city of Peoria to sell, offer, or expose for sale, any animal, fowl, or game used for food purposes, refrigerated or otherwise, which has not been properly drawn and prepared by removing the viscera (bowels, entrails) at the time of slaughter.

SEC. 2. Any person, firm, or corporation violating any of the provisions of this ordinance shall be deemed guilty of a misdemeanor, and upon conviction thereof shall be punished by a fine not exceeding one hundred dollars.

PROPOSED LEGISLATION IN NEW YORK.

In the State of New York in January, 1907, there was offered the following amendment to Section 165 of the agricultural law, defining certain foods the sale of which is prohibited.

If it consists of any slaughtered game, animal, poultry or fowl, unless the carcasses of such slaughtered game, animal, poultry, or fowl shall have been divested of its lung tissues, entire digestive and intestinal tracts, gall receptacle, craw, and gizzard lining, within twelve hours after its slaughter; and any slaughtered game, animal, poultry, or fowl found in any refrigerator, ice chest, cooler, storage apartment, or market, whether exposed for sale, or in stock, shall be presumed to have been slaughtered for a longer period than twelve hours.

This amendment has been referred to under the discussion of the work of Doctor Cavana and Doctor Higley, respectively.

PROPOSED LEGISLATION IN THE DISTRICT OF COLUMBIA.

There was presented to the Fifty-ninth Congress on March 12, 1906, a bill relating to the sale of poultry in the District of Columbia, which bill recommended that the shipping of any poultry into said District, intended for sale, refrigerated or otherwise, which has not been properly drawn and prepared by removing the viscera at the time of slaughter shall be considered unlawful, a fine and imprisonment being provided as a punishment for the violation of this act. In the report of the committee to which this bill was referred there is a recommendation that it be passed.

CANADIAN COLD-STORAGE ACT.

An interesting contribution to the subject of cold storage is found in the report of the dairy and cold storage commissioner of the Dominion of Canada for the year ending March 31, 1907. The Dominion of Canada has already secured the enactment of a law

relating to the cold storage and regulations thereunder which are of interest in this connection. Certain portions of the act and regulations are as follows:

THE COLD-STORAGE ACT.

An act to encourage the establishment of cold-storage warehouses for the preservation of perishable food products.

His Majesty, by and with the advice and consent of the Senate and House of Commons of Canada, enacts as follows:

1. This act may be cited as the Cold Storage Act.

2. The Governor in Council may enter into contracts with any person for the construction, equipment, and maintenance in good and efficient working order of public cold-storage warehouses equipped with mechanical refrigeration, in Canada, and suitable for the preservation of all food products.

3. The location, plans, and specifications of every such warehouse, its equipment, and the amount to be expended thereon shall be subject to the approval of the Governor in Council.

4. The Governor in Council may, out of any moneys appropriated by Parliament for the purpose, grant toward the construction and equipment of any such warehouse a subsidy not exceeding in the whole 30 per cent of the amount expended or approved of in such construction and equipment, and payable in installments as follows: Upon the warehouse being completed and cold storage at suitable temperatures being provided therein, all to the satisfaction of the Minister of Agriculture, a sum not exceeding 15 per cent of the amount so expended; and at the end of the first year thereafter 7 per cent of the said amount; at the end of the second year thereafter, 4 per cent of the said amount, and at the end of each of the two next succeeding years, 2 per cent of the said amount: *Provided*, The warehouse is maintained and operated to the satisfaction of the Minister of Agriculture.

5. The Minister of Agriculture may refuse to pay any part of the said subsidy if, in his opinion, the operation of the warehouse has not been of such a character as to provide for the proper preservation of such products as may be stored therein.

6. The Minister of Agriculture may order, and cause to be maintained, an inspection and supervision of the sanitary conditions, maintenance, and operation of such warehouses, and may regulate and control the temperatures to be maintained therein in accordance with the regulations to be made as hereinafter provided.

7. The rates and tolls to be charged for storage in such warehouses shall be subject to the approval of the Governor in Council.

8. For the effective carrying out of the provisions of this Act the Minister of Agriculture may appoint inspectors, who shall have access to all parts of such warehouses at all times.

9. The Governor in Council may make such regulations as he considers necessary in order to secure the efficient enforcement and operation of this Act; and he may by such regulations impose penalties not exceeding fifty dollars on any person offending against them; and the regulations so made shall be in force from the date of their publication in the *Canada Gazette*, or from such other date as is specified in the proclamation in that behalf.

Among the regulations for the enforcement and operation of this act are the following:

2. No applications shall be considered for any cold-storage warehouses except those equipped with mechanical refrigeration, nor for any place where any such cold storage already exists or where the proposed cold storage would compete directly with other establishments of the same class. * * *

4. The owners of cold-storage warehouses, in order to secure the subsidy, will be required to maintain the following temperatures therein for the preservation of the various products mentioned:

Kinds of produce.	Temperature (° F.).	
	Minimum.	Maximum.
Apples and other fruits	32	36
Butter		20
Cheese	35	40
Eggs, meats, and dressed poultry	30	34
Bacon and hams	40	45
Fish (frozen)		20
Meats, poultry, and game (frozen)		20
Vegetables	34	38

5. Nothing in these regulations shall prevent owners of subsidized cold-storage warehouses from entering into special contracts with customers for the maintenance of temperatures other than those herein specified. * * *

7. The owners of cold-storage warehouses, to which the subsidy or any part thereof has been paid, may be required to make an annual report to the Minister of Agriculture in such form as may be prescribed.

The subject of cold storage is of great importance in Canada, particularly in relation to the export of food products. The rapid increase of population in the western part of the Dominion, where the production of perishable products of the farm is not large enough to supply the local needs, has, however, developed a great internal trade in cold-storage products. It is said by the Commissioner on page 156 of his report:

Cold storage enables the dairyman to store his surplus butter and cheese during the active season of production and to dispose of it during the off season, when manufacture has almost, if not entirely, ceased. Prices are equalized, and the consumer is supplied with an article in better condition throughout the whole year than would otherwise be possible. Not only is the business of the producer enormously increased, but the commerce of the country derives a corresponding benefit.

The Dominion of Canada has encouraged the industry of cold storage by offering certain subsidies, as is provided for in the bill preceding. The dairy and cold storage commissioner says in regard to this matter:

The policy of giving financial assistance toward the erection of public cold-storage warehouses in Canada was adopted by the Government during the session of 1906–7 by the introduction of the Cold Storage Bill, entitled an "Act to encourage the establishment of cold-storage warehouses for the preservation of perishable food products." Parliament approved of the measure, and it became law without delay.

The requirements of trade in certain localities have made cold storage an absolute necessity, and in such places the revenue is sure enough to make the investment a fairly safe one, so that it needed no special inducement to secure the capital required to provide the necessary facilities.

While it may, at first glance, seem a little unfair to assist the new enterprises, even if noncompetitive, it must be borne in mind that the existing cold-storage warehouses occupy the choice locations, and for that reason some inducement seems to be necessary if similar facilities are to be provided in other localities.

There is a large quantity of perishable produce handled in this country without cold storage, the value and stability of which would be much more improved by its use. Where it is possible to get along without cold storage, even if the results are unsatisfactory, the question is not studied so closely, and the improved facilities come more slowly than is the case where the necessities are greater. It is hoped that the attention which has been drawn to the subject by the adoption of the principles involved in the Cold Storage Act, and the discussion which will naturally arise thereon, will have an important educational influence in the direction of creating a more general appreciation of the advantages of cold storage and a greater demand for such facilities. It is believed also that this process of education will result in bringing more business to existing cold-storage warehouses.

It will be seen by an inspection of the quotations made from the report and comments thereon that the object of the regulation of cold storage in Canada is more for the development of commerce than it is to study the effects of cold storage upon the wholesomeness of the products. Nevertheless, sanitary provisions are included in the act in section 5, where the subsidy which is offered may be refused if precautions for the proper preservation of the products have not been observed, and also in section 6, where a sanitary inspection and supervision of the cold-storage plants are provided for. The Dominion of Canada, it appears, has taken a more active interest officially in cold storage than any other country so far as is known, since they have recognized it by an act of Parliament and provided to a certain extent for its regulation.

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